

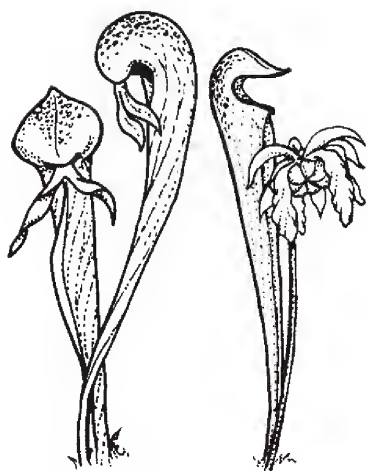
CARNIVOROUS PLANT NEWSLETTER

Journal of the International Carnivorous Plant Society

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Front Cover: *Pinguicula megaspilaea* growing on serpentine rock in Muğla province, Turkey. Photo by Andreas Fleischmann. Article on page 48.

Back Cover: Dry landscape stretches for kilometers around this *Darlingtonia* site in southern Oregon. This is the challenge which *Darlingtonia* must overcome to colonize new habitat. Photo by Damon Collingsworth. Article on page 44.

Inside Back Cover: A dense population of *Darlingtonia* on a rainy day in southern Oregon. Photo by Damon Collingsworth. Article on page 44.

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CPN EDITORS

In April 1972, Don Schnell and Joe Mazrimas worked together to release the first Carnivorous Plant Newsletter – a 15-page mimeograph. In 1978, they were joined by Leo Song and Larry Mellichamp and some color pages were added.

In 1997, editorial leadership shifted to Barry Rice and Jan Schlauer. Steve Baker conducted the page layout. In 2009, Stephen Davis took over the role of managing editor. In 2010, Bob Ziemer became managing editor with assistance from Barry Rice, Jan Schlauer, and Fernando Rivadavia. In 2014, Djoni Crawford joined the editorial staff. The entire staff works on a volunteer basis.

On July 19, 2014, five of CPN’s past and present editors attended the annual BBQ hosted by California Carnivores: (from left) Bob Ziemer, Barry Rice, Leo Song, Steven Davis, and Joe Mazrimas.

Editors	Tenure
Joe Mazrimas	1972-1998
Don Schnell	1972-1996
Leo Song	1978-1993
Larry Mellichamp	1978-1987
Steve Baker	1994-2008
Barry Rice	1997-2015
Jan Schlauer	1997-2015
Tony Gridley	2009
Steven Davis	2009-2010
Bob Ziemer	2009-2015
Fernando Rivadavia	2009-2015
Djoni Crawford	2014-2015



DO BEARS DISPERSE *DARLINGTONIA*?

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Sodden fields of silent green serpent heads complete with dark red tongues stretch out before me as I explore the Northern California habitat of *Darlingtonia californica* – the Cobra Lily. Closer examination of their domed heads reveals the clear patches or fenestrations which trick insects into following the light down into the pitchers. As I marvel at these fascinating plants, I ask myself rhetorically, “How could they be any more interesting?” Well, what if their seeds were distributed by bears?

This isn’t exactly a new idea, but it hasn’t been widely accepted as fact either. Peter D’Amato has long suspected this to be true. He even mentioned it in the first edition of his book, “The Savage Garden”. Peter notes, “the small seeds are bristly, no doubt to encourage dispersal by animals.”

Indeed, the little club-shaped seeds are covered in tiny bristles that immediately make you think of animal dispersal. Bears are an obvious suspect because of their long, shaggy fur and love of water.

In 2003 Harry Tryon took Peter and me to a *Darlingtonia* site that he calls “bear wallows” in Del Norte County, California. His red pickup truck jostled us down narrow timber “roads” composed of loose rock, a crumbling uphill slope to the right, and a sheer drop of at least 50 meters to the left. Peter leaned in from the back seat and asked, “If we fell how long do you think it would take to find our bodies?”



Figure 1: Harry Tryon leads the way to “Bear Wallows”, with a *Darlingtonia*-lined wallow beyond him; and an American black bear (*Ursus americanus*). Left photo by Damon Collingsworth; right photo by U.S. Fish and Wildlife Service.

Shortly after the uneasy laughter died down, the truck thankfully came to a stop. As we got out, Harry pointed out bear tracks and warned us. He said that he never visits this spot by himself. We followed him quietly down a trail to the wallows. I noticed bear hair stuck on the low branches and bark of the tree trunks as I walked. The trail led us downhill a ways and ended in front of a bear-sized pool bordered by Cobra plants. Our path literally dumped into the wallow and then climbed out on the other side before dropping into another cool pool. As I recall, the path led through at least three more of these small pools, each surrounded by *Darlingtonia*. Thankfully the wallows were not in use at the time, but their purpose was clear. Bears must surely take this path to cool down. It was an amazing site where, probably due to the relatively low light, the *Darlingtonia* grow pitchers that are about waist high. While this evidence is admittedly largely circumstantial it does lend some credence to the idea that the seed were hitchhiking on the bears to find areas to germinate where the water can cool the plants' roots on hot summer days.

Years later, after a *Darlingtonia* talk I gave at the 2012 ICPS conference, Dr. Adam Cross approached me. He was quite intrigued by the bear idea in particular and offered to do a SEM image of *Darlingtonia* seed. He has done quite a bit of research on seed dispersal in Australia and so his insights have been invaluable. When I received the initial SEM images of the seed (Fig. 2), I was a little disheartened. The bristles lacked barbs or hooks that might more firmly suggest animal dispersal. In personal communication with Adam, he pointed out that the dispersal objective of *Darlingtonia* is very unique, which might account for the somewhat tame look of the bristles. It has been pointed out by others that the bristles would also add surface area to the seed allowing them to float better on the surface of water. This might suggest that the bristles are purely for water dispersal, but given the vast expanses of hot and dry landscapes that *Darlingtonia* seeds have traveled to find the cool streams and fens where they grow, it seems that water dispersal certainly cannot be the whole story.

Adam and I would point out here that the ideal dispersal model for *Darlingtonia* would be for the seeds to be bristled just enough to hang in the shaggy hair of a wandering bear, but not so hooked as to get stuck permanently in their fur. Bears are wallowers, so *Darlingtonia* seeds deposited in their fur would have a good chance of finding their way into some water. Once in the water, however, the seeds will need to release from the hair into the water and float to some suitable location for germination. We believe it is this two-step distribution that explains why the seed don't look more aggressive when compared to other more typically animal-distributed seeds.

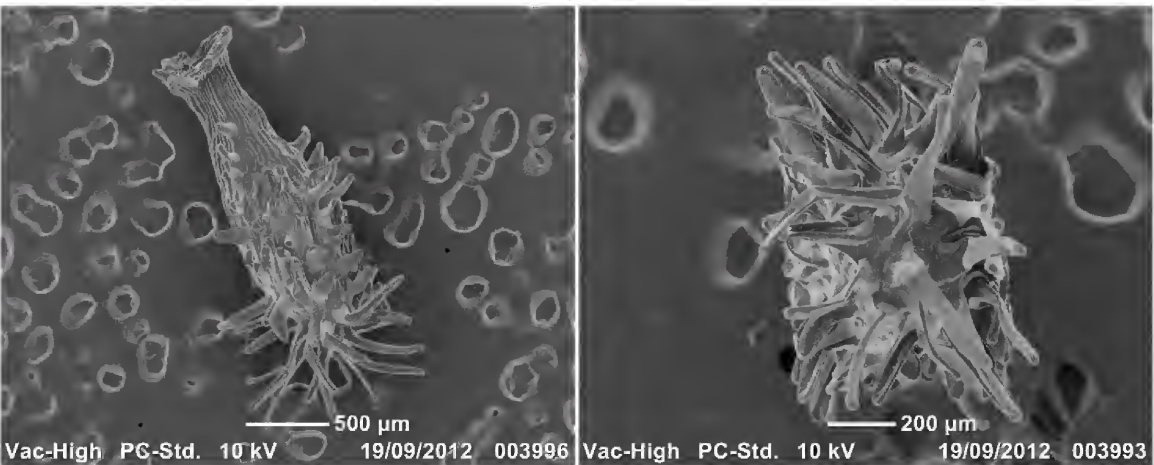


Figure 2: A close look at the seed coat projections which enable the seeds to entangle in the hair of animals. Photo by Adam Cross.



Figure 3: The full progression of *Darlingtonia* flowers, starting with the freshly opened flower in the left background. In the foreground from bottom to top, each flower shows how the flowers turn up after pollination; and on the right, a nearly ripened seed pod fully upturned.

I've spent quite a bit of time walking amidst the Cobra plants now and I have noticed another trait that further supports this theory of bear-dispersal. Like *Sarracenia*, *Darlingtonia* flowers are born on tall scapes and they hang like little green and purple lanterns. Once the flower is pollinated, the scape straightens out turning the fruit upside down before it dehisces. By autumn, the fruit cracks open slightly longitudinally, but because the fruit is upturned like a cup, nearly all of the seeds are stuck inside. This would be a pretty big evolutionary flaw for a plant family which is so specialized and evolved. The common sense of nature dictates that there must be some evolutionary advantage to this. *Sarracenia flava* and *S. leucophylla* flowers often turn up after pollination, but this helps the seeds to fall away from the umbeli-



Figure 4: A fully opened seed pod from above showing the seeds still trapped inside. Photo by Jason Ksepka.

form pistil. In *Sarracenia*, the upturned pistil can also shade the developing fruit from the scorching southern sun after the flower has inverted.

So, why do *Darlingtonia* flowers then seem to stupidly invert and trap their seeds in their own fruit? It would take less energy to leave the flower the way it is and just drop the seed in the water below if water dispersal was your sole goal.

After thinking about this long and hard, I propose that the seeds are not stuck, but waiting for a ride. A very few seeds often do immediately fall from the openings in the side of the dried fruit and might very well disperse a short distance in the water below. Almost all of the other seeds are still stuck in the open fruit even a year after they've opened. You often see last year's pods still full of old seed. I have noticed that as I troop through the Cobra plants, I brush past the tall, stiff scapes. They would be pushed down slightly, then spring back to attention, flinging the seeds everywhere. No doubt, as bears lumber through the water-logged plants the seed would be flung into their fur in the same way. Obviously, seeds that have fallen on the ground are much less likely to casually make their way into the fur of an animal, making the type of long distance dispersal necessary for finding new habitat unlikely at best. Strangely then, falling immediately out of the fruit becomes evolutionarily limiting if not truly disadvantageous.

Although, water is an obvious part of *Darlingtonia*'s seed dispersal strategy, particularly within suitable habitat once colonized, animal dispersal is almost certainly key to colonizing new suitable habitat. Miles and miles of dry rocky hills often separate *Darlingtonia* sites. How else would their seeds traverse all of this unsuitable land to find their very specific and often isolated habitat?

In comparison, the most likely mechanism for *Sarracenia* colonization of new habitat is almost certainly wind, despite their seeds' rather rotund appearance. The southeastern United States is infamous for both hurricanes and tornadoes, but they can occur along the entire eastern seaboard of the U.S. The Atlantic hurricane season starts in July and ends in November. *Sarracenia* seeds usually mature by late August and pods are fully dehiscent by late September to early October. A Category 1 hurricane has wind speeds of 119-153 km/h and a Category 5 has winds >252 km/h. Tornadoes often occur within the hurricanes. A very weak tornado starts at 105 km/h and the biggest have winds exceeding 320 km/h. With severe weather like this, it is not hard to imagine how little round *Sarracenia* seeds, seed pods, or indeed entire plants might be scattered to every wet corner of the southeast. Especially when you combine that with what used to be a more contiguously wet region than California. Thankfully for us, the cold waters of the Pacific Ocean prevents strong hurricanes from hitting Northern California. Strong winds can occur, but these major wind events are very rare with the worst wind usually at high elevation. *Darlingtonia* are more commonly found in wet areas between the hills and mountains where they would be mostly protected from strong winds. The few higher elevation populations would almost always be buried by snow before or during these storms, as California only has strong storms in the winter. This all makes significant wind dispersal of *Darlingtonia* seed improbable at best.

Due to the lack of credible fossil record of the family Sarraceniaceae, it is unclear where they initially evolved or even when *Darlingtonia* branched off. Regardless of the details, I think that a long-distance dispersal event via animals could be responsible for *Darlingtonia*'s far-flung range relative to *Sarracenia*. Indeed, it could have been that slight difference in the seed coat that carried the family to California where *Darlingtonia* diverged due to the extreme and long geographical isolation. Granted this is conjecture, but it is fun to think about.

THE INTRICATE *Pinguicula crystallina/hirtiflora*-COMPLEX

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Keywords: Lentibulariaceae, *Pinguicula*, Mediterranean, Anatolia, cultivation.

The *Pinguicula crystallina/hirtiflora*-species-aggregate (*Pinguicula* section *Cardiophyllum*, which will be further simply called the “*P. crystallina*-complex” here) comprises rosetted perennial temperate butterworts of homophyllous growth type (the latter means that carnivorous leaves are produced throughout the year, no hibernacula or non-carnivorous winter leaves are present, and the plants hibernate with open leaf rosettes) that are distributed in the Mediterranean (southern Italy, the Balkans, and Greece) and Asia Minor (Cyprus and Turkey).

Taxonomic history and difficulties of this intricate group of *Pinguicula* have been dealt in detail by Ernst (1961), Casper (1962, 1966, 1970, 2004, 2006), Peruzzi (2006), and Shuka *et al.* (2007), yet still many questions remained regarding species concepts and circumscriptions. The species-complex is reviewed here, based on newly available data on distribution, morphological plasticity, and chromosome numbers, based on own field and herbarium studies, as well as on data gained from plants in cultivation.

Four morphologically quite distinct taxa can be distinguished in this natural affinity, based on corolla shape, and leaf shape and size. Biogeography, size and ultrastructure of the seeds, as well as cytology (diploids and tetraploids, based on a common chromosome base number of $x = 14$; Casper & Stimper 2009) generally support these four lineages. In accordance, three of the four taxa of this complex are treated here as separate species, one on variety rank: *Pinguicula crystallina*, *P. hirtiflora* var. *hirtiflora*, *P. hirtiflora* var. *louisii*, and *P. megaspilaea*. A comparative treatment of these four taxa is given below.

Material and Methods

Information about distribution, habitats, as well as morphological data is based on examination of herbarium specimens and digitized images thereof (AIX, B, G, K, M, MARS, P, WU), on own observations of plants *in situ* and in cultivation, as well as extracted from literature (i.e. all sources listed under “References”).

Identification key to the species of the *P. crystallina* -complex:

1. Lobes of corolla upper lip distinctly smaller (shorter and narrower) than those of the lower lip, spur 4-6(-8) mm long (1/3 of the corolla length) ... *P. crystallina*
 - Lobes of corolla upper and lower lip (sub)equal in size, spur longer than 6 mm (usually 7-20 mm long, comprising more than half of the corolla length) ... 2
2. Lobes of the lower and upper corolla lip rounded, obtuse or slightly truncate; fully grown summer leaves usually with reflexed margins (the very margin only slightly enrolled); leaves of summer growth usually half-erect ... 3
 - Lobes of the lower (and usually also upper) corolla lip notably truncate to emarginate; leaves with distinctly enrolled margins; leaves of summer growth usually prostrate on the ground ... *P. hirtiflora* var. *hirtiflora*
3. Leaves of summer growth 1-2 cm long (rarely up to 8 cm), oblong-elliptic to obovate; spur (8-)13-22 mm long, equaling to exceeding the length of the rest of the corolla ... *P. hirtiflora* var. *louisii*

- Leaves of summer growth 4-12 cm long, very narrowly (ob)lanceolate to linear, their very margins reflexed; spur 6-11 mm long, notably shorter than the rest of the corolla (tube + spread lips) ... *P. megaspilaea*

Pinguicula crystallina Sm.

Leaves 15-25(-40) mm long, 10-25 mm wide, broadly elliptic to oblong, with distinctly enrolled margins and emarginate or obtuse apex; “winter leaves” only slightly smaller; leaves yellowish green or (rarely) tinged purple-reddish. *Scapes* 30-60(-100) mm long, densely glandular. *Calyx* bilabiate, upper lip trilobate (sepals free almost to the base), lower lip bilobate, lobes fused for half or more of their length, with obtuse apices. *Corolla* 8-15(-21) mm long (including spur), bilabiate, upper and lower lip forming an angle of ca. 90° or more, corolla white, each corolla lobe with violet-blue tip (sometimes lobes entirely white), corolla throat and spur yellowish-green, throat with few reddish-brown stripes; upper lip two-lobed, lobes widely divergent, with obtuse apex; lower trilobed, lobes spreading or slightly overlapping, each with obtuse to slightly truncate apex; spur narrowly cylindrical, (3-)4-6(-8) mm long, slightly curved downwards or straight. *Seeds* 0.7-0.85 mm long, cylindrical to ellipsoidal, testa reticulate (Mikeladse 1996).

Chromosome number: $2n = 28$ (Mikeladse 1996; Mikeladse & Casper 1997; Casper & Stimper 2004, 2006, 2009; Casper *et al.* 2007, all for material from Cyprus).

Etymology: The epithet *crystallina* (Lat. “of crystal”) was chosen by the species’ author, James Edward Smith, because of “the clear drops on its leaves glittering like diamonds in the sunshine” (Holmboe 1914).

Distribution: Cyprus, Turkey (Fig. 1). On Cyprus limited to the central part of the Troodos Mountains of Western Cyprus (seven populations are known from the Troodos Forest National Park; Georgioui *et al.* 2007), however an outlying lowland population has recently been discovered in

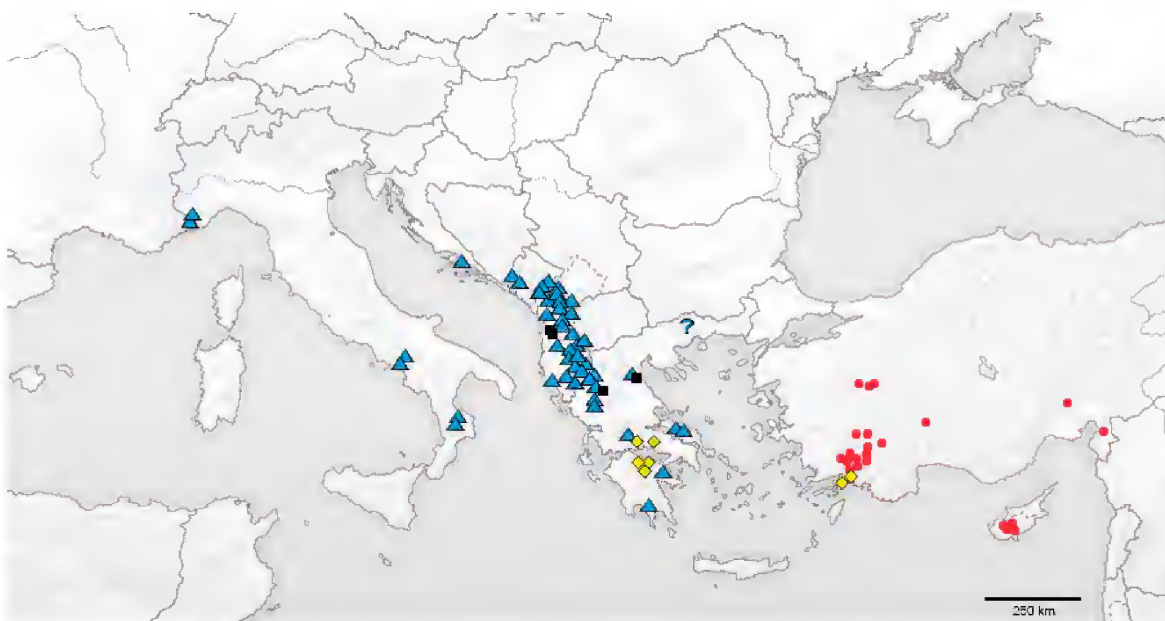


Figure 1: The distribution of the *P. crystallina*-complex in the Mediterranean and Asia Minor. *P. crystallina* (red dots), *P. hirtiflora* var. *hirtiflora* (blue triangles), *P. hirtiflora* var. *lousiis* (black squares), and *P. megaspilaea* (yellow diamonds). The blue question mark refers to a doubtful literature record of *P. hirtiflora* from the Falakro Mountain of NE Greece.

the Lemesos Forest (Christodoulou 2006). In Turkey, the species occurs widespread, yet localized, in SW Anatolia (Mill 1978), but also in disjunct populations in S Anatolia (Adana and Hatay provinces; Yildirim *et al.* 2012), the latter in the south-east of the country close to the Syrian border (foothills of the Nur Dağları Mountains; Adamec 1996, 1997; Adamec & Pasek 2000).

Habitat: On Cyprus at 800-1640(1900) m elevation in the Troodos Mountains (Casper 1970; Steiger 1998; Georghiou *et al.* 2007), but the outlying population in the Lemesos Forest at just 250 m altitude (Christodoulou 2006). In Turkey in montane regions (1000-1700 m; Casper 1970), but again as low as ca. 250 m in canyons of the foothills of the Nur Dağları Mountains in SE Turkey (Adamec 1997). The species is confined to permanently wet habitats, where it grows as a lithophyte on wet rocks (Fig. 2), near springs, streams, and waterfalls, but also in peat soil in boggy seepage habitats. Usually found growing over serpentine rock (e.g. Cyprus, Sandras Mountains of Turkey), but also on limestone (e.g. mountains of Denizli province, Turkey), and in SE Turkey on basaltic rock associated with very alkaline water (Adamec 1996, 1997; Adamec & Pasek 2000). The high altitude populations of this species hibernate as open, carnivorous leaf rosettes covered by snow and ice.

Conservation status: Vulnerable on Cyprus (only eight populations are known from the island) and also vulnerable in Turkey, where the species is more widespread, but occurs in localized populations. Populations both on Cyprus and in Turkey are under potential threat by human influence on the hydrology (e.g. by spring capture or channeling), by road construction, but also by natural drought (Georghiou *et al.* 2007). Ironically, at least on paper, the species is strictly protected by the Bern Convention (Convention on the Conservation of European Wildlife and Natural Habitats, Appendix I).

Notes: Some of the previously used characters to distinguish *P. crystallina* from *P. hirtiflora* have proven unreliable, such as the shape and size of the leaves, the shape of the leaf tip (emarginate vs.



Figure 2: *Pinguicula crystallina* growing in the Troodos Mountains of Cyprus. Photos by Stewart McPherson.



Figure 3: *Pinguicula crystallina* in cultivation. Top: Plants from Mount Güneğ, Denizli, Turkey (naturally growing on limestone). Bottom: Plants from Sandras Mountains, Muğla, Turkey (naturally growing on serpentine).

obtuse), or the indumentum inside the corolla throat (see Casper 1970), or the calyx shape, which is not different between the two species (Schindler 1908).

However the shape of the corolla, the length of the tubular part of the corolla, and the size of the spur allow readily to distinguish both taxa (Fig. 2, 3). Regarding the obvious close relationship of these two taxa, the question arose whether they should indeed be kept as distinct species, or if they are best considered two lineages of a single species. The latter has been put forward by Strid (1991), who treated the two species as geographically separated subspecies. Here, the “classical” approach (e.g. Schindler 1908; Ernst 1961; Casper 1966, 1970; Mikeladse & Casper 1997) to maintain both taxa as separate species is applied: the Anatolian-Cypriot *P. crystallina* and the Italian-Balkan *P. hirtiflora* – there is no overlap evident in the range of the two species (see Fig. 1). Moreover, an artificially created hybrid between both species (using *P. hirtiflora* var. *hirtiflora* as the pollen donor and *P. crystallina* from Cyprus as the mother plant) proved to be male sterile (pollen is malformed, despite complementary chromosome numbers of both parent species), which indicates not only a geographical, but also a reproductive separation of both species. Interestingly, the hybrid resembles *P. hirtiflora* var. *hirtiflora* more in the vegetative parts, while the flowers show most characters of *P. crystallina*, such as a more tubular corolla with very short upper lip (Fig. 4).

Pinguicula megaspilaea Boiss. & Heldr. ex Boiss.

Synonyms: *P. hirtiflora* var. *megaspilaea* (Boiss. & Heldr. ex Boiss.) Schindler, *P. hirtiflora* subsp. *megaspilaea* (Boiss. & Heldr. ex Boiss.) Nyman, *P. hirtiflora* var. *gionae* Contandr. & Quezel, *P. habilitii* Yıldırım, Şenol & Pirhan; as “*P. hirtiflora* var. ?” in Casper *et al.* 2007

Leaves during anthesis (40-)70-120 mm long, 5-11 mm wide, very narrowly oblanceolate (lanceolate in the Turkish plants) to linear, usually with backwardly reflexed margins; “winter leaves” much shorter and slightly wider, 18-35 mm long, 11-16 mm wide, oblong to oblanceolate, with slightly enrolled margins; leaves yellowish green or tinged purple-reddish. *Scapes* 110-170 mm long, densely glandular. *Calyx* bilabiate, upper lip trilobate (sepals free almost to the base), lower



Figure 4: Artificially created hybrid between *P. hirtiflora* var. *hirtiflora* and *P. crystallina*, grown in the author's greenhouse. The plant does not produce viable pollen, but readily propagates by division of the mother rosette.

lobe entire (consisting of two fully fused sepals) with obtuse to truncate apex. *Corolla* 22-28 mm long (including spur), bilabiate, upper and lower lip spreading at ca. 180° (forming one line), corolla white, each corolla lobe with violet blue tip, corolla throat and spur yellowish-green, throat sometimes with few reddish-brown stripes; upper lip two-lobed, lobes widely divergent, with obtuse apex; lower lip trilobed, lobes slightly overlapping, each with obtuse to slightly truncate apex; spur narrowly cylindrical, 6-11 mm long, tapering towards acute apex. *Seeds* comparatively large and wide (compared to the other three members of the affinity), ca. 1 mm long, oblong to narrowly elliptical, testa reticulate (Yildirim *et al.* 2012).

Chromosome number: $2n = 56$ (Casper & Stimpert 2006, 2009 and Casper *et al.* 2007, for material from Styx and Reka Gorge, Greece; haploid karyotype of Turkish plants $n = 28$, obtained from pollen mother cells from fixed flower buds of cultivated material, following the protocol from Casper & Stimpert 2009). Contandriopoulos & Quezel (1974) counted $2n = 48$ from material from Styx, Greece, however this karyotype was put in question by Shuka *et al.* (2007) and Casper *et al.* (2007). This species has the double chromosome number of *P. hirtiflora*, hence could represent a tetraploid lineage.

Etymology: The epithet refers to the Megaspilaeon (or: Mega Spileon, Greek for “big cave”) Monastery in the Chelmos Mountains, Peloponnese, Greece, where this species was first collected.

Distribution: Greece (Peloponnese: Chelmos Mountains, and Central Greece: Mt. Giona and Mt. Parnassus; Casper *et al.* 2007, from the latter localities reported as *P. hirtiflora* var. *gionae* and “*P. hirtiflora* var. ?”) and Turkey (known from a single population in Marmaris province (the type locality of *P. habillii*; Yildirim *et al.* 2012), as well as from a recently discovered second locality in Muğla province (pers. obs.)). This species shows an interesting disjunction, from the mountains of central Greece to the coastal region of SW Turkey (Fig. 1).

Habitat: Found growing lithophytically on serpentine rock at 80-200 m in SW Anatolia, Turkey (Yildirim *et al.* 2012; pers. obs.; Fig. 5). However, in Greece also on limestone rock and known from 650 m to up to 1930 m altitude (Contandriopoulos & Quezel 1974; Casper *et al.* 2007; J. Schlauer, pers. comms.). The populations in SW Anatolia are geographically (especially altitudinally) well-separated from the nearest known populations of *P. crystallina* (Yildirim *et al.* 2012; pers. obs.).

Conservation status: Considered critically endangered in Turkey due to human development (channeling, road construction; Yildirim *et al.* 2012). The few known populations in Greece are also best classified as endangered, as they are in potential threat by spring channeling (J. Schlauer, pers. comms.).

Notes: The plants described as *P. hirtiflora* var. *gionae* from Greece by Contandriopoulos & Quezel (1974), agree morphologically (and cytologically: $2n = 56$, Casper & Stimpert 2006) with *P. megaspilaea*, and are hence included in this species here.



Figure 5: *Pinguicula megaspilaea* growing on serpentine rock in Muğla province, Turkey. This is the second known location of this taxon from Turkey, where it has previously been described as *P. habillii* from Marmaris province.

This species differs much from the other members of the complex by producing long, upright leaves with reflexed margins during summer growth (Fig. 5; these “thread-like” leaves resemble those of the only distantly related *P. longifolia*). Elongate summer leaves are especially found in plants growing lithophytically on vertical rock – plants growing in boggy habitats (e.g. in the upper Styx valley) have leaves that are more similar to those of *P. hirtiflora* (J. Schlauer, pers. comms.). In terms of flower morphology, it is quite similar to *P. hirtiflora* (see Schindler 1908), except that *P. megaspilaea* usually has the tips of its corolla lobes entire and rounded (see Fig. 5), while these are usually emarginate in *P. hirtiflora*.

The “heterophyllous growth” which Yildirim *et al.* (2012) mention for their *P. habillii* does not correspond to the term “heterophyllous” as applied by Casper (1966) for his classification of *Pinguicula* growth types. The authors refer to the conspicuously longer, narrower carnivorous leaves produced during flowering time (“summer leaves”), compared to the much smaller, albeit still carnivorous “winter leaves” of this species – that means it is an anisophyllous species. A similar decrease of rosette diameter in autumn/winter can be observed in any of the three other members that belong to this affinity (*P.* section *Cardiophyllum*), too, as all of them produce (slightly to distinctly) smaller carnivorous leaves during this time. Nevertheless, all of them classify as the “temperate homophyllous growth type” as defined by Casper (1966), as continuous growth and production of carnivorous foliage occurs throughout the year.

Pinguicula hirtiflora var. *louisii* (Markgr.) A.Ernst

Synonyms: *P. louisii* Markgr., *P. hirtiflora* var. *decipiens* Bornm.

Leaves 10-20(-80) mm long, 6-10(-25) mm wide, oblong-elliptic to obovate, with just minutely enrolled margins, yellowish green or tinged purple-reddish (especially near the base). *Scapes* (30-) 40-150 mm long, sparsely glandular, glabrous towards the base. *Calyx* bilabiate, upper lip trilobate (sepals free almost to the base), lower lobe entire (consisting of two fully fused sepals), with obtuse or retuse, rarely emarginate, apex. *Corolla* 22-29 mm long (including the very long spur), bilabiate, upper and lower lip forming an angle of ca. 80°-110° (Casper 2004), corolla white, each corolla lobe with bright to pale violet-blue tip, corolla throat and spur yellowish-green, throat with few reddish-brown stripes; upper two-lobed, lobes divergent, or slightly overlapping, with rounded apex; lower lip trilobed, lobes oblong to almost orbicular, each lobe with rounded to only slightly



Figure 6: *Pinguicula hirtiflora* var. *lousii* growing at Mount Olympus, Thessaly, Greece. Photos by Christian Klein.

truncate apex; spur narrowly cylindrical, (8-)13-20(-22) mm long, straight or only slightly curved downwards, tapering towards acute apex. *Seeds* 0.6-0.8 mm long, cylindrical to ellipsoidal, testa reticulate (Shuka *et al.* 2007).

Etymology: This taxon was originally named as a species, *P. lousii*, in honor of geographer Dr. H. Louis from Berlin, who took part on some of Markgraf's Albanian expeditions (Markgraf 1926; Casper 2004).

Distribution: In contrast to Casper (2004) and Shuka *et al.* (2007), who considered *P. hirtiflora* var. *lousii* (or *P. lousii*, respectively) to be narrowly endemic to central Albania, the author of the present work has studied material from Mount Olympus, Greece, and agrees with Markgraf (1926) that some of these populations also morphologically fall within the range of that taxon. However, *P. hirtiflora* var. *hirtiflora* also occurs on the large Olympus mountain massif (see e.g. Casper *et al.* 2007), but apparently never sympatrically with var. *lousii*, like this is also the case in Albania. Peruzzi (2007) additionally reported and pictured the variety from N Pindhos, Greece (under the name "*P. hirtiflora*"). The range of *P. hirtiflora* var. *lousii* is fully included within the Balkan range of *P. hirtiflora* var. *hirtiflora* (Fig. 1).

Habitat: In wet calcareous meadows or on steep seeping slopes in Albania (Markgraf 1926; Casper 2004; Shuka *et al.* 2007), on limestone rocks near springs on Mt. Olympus, Greece (Ch. Klein, pers. comms.; Fig. 6). At lower altitudes of 150-700(-900) m in Albania (Shuka *et al.* 2007), at ca. 1000-1500 m on Mt. Olympus.

Conservation status: Considered endangered in Albania, as only three populations are known, all in close proximity to cities, and one known site apparently is extinct now due to human water constructions (Shuka *et al.* 2007, in their footnote 34). The known populations at Mt. Olympus, Greece, lie within the borders of a National Park.

Notes: *Pinguicula hirtiflora* var. *lousii* has sometimes been treated as distinct species, *P. lousii* (Markgraf 1926; Casper 2004). Although it differs morphologically from *P. hirtiflora* var. *hirtiflora* regarding corolla shape (maybe as much as *P. crystallina* differs from *P. hirtiflora*), this certainly is the taxonomically most tenuous member of the complex, as there is full geographical and some morphological overlap with *P. hirtiflora* var. *hirtiflora*; however, this taxon seems to be separated at least cytologically (being a tetraploid) in most populations. Also, the flowers of *P. hirtiflora* var. *lousii*, with entire rounded margins of the corolla lobes and a comparatively long, acute spur (Fig. 7) will generally help to distinguish it from *P. hirtiflora* var. *hirtiflora*. Hence the opinion of Ernst (1961), Casper (1962, 1966), and Shuka *et al.* (2007) is followed here to treat this taxon as a separate



Figure 7: *Pinguicula hirtiflora* var. *lousiiei* in cultivation. Top: plants from Linza, Albania. Note the comparatively long spur of this variety, as well as the narrow corolla lobes with rounded entire margins. Bottom: Exact locality unknown, “former Yugoslavia”, plants from BG Munich (most likely from a location in Albania).

variety. Even the species’ original author, Markgraf, later decided to finally follow the recombination of Ernst (1961), and regarded his *P. lousiiei* as a variety of *P. hirtiflora* (Shuka *et al.* 2007).

Pinguicula hirtiflora Ten. var. *hirtiflora*

Synonyms: *P. crystallina* subsp. *hirtiflora* (Ten.) Strid, *P. hirtiflora* var. *euboea* Beauverd & Topali, *P. hirtiflora* f. *pallida* Casper, *P. albanica* Griseb., *P. laeta* Pant., *P. lavalvae* Innangi & Izzo (in press), *P. vulgaris* var. *hirtiflora* (Ten.) Ces., Pass. & Gibelli

Leaves (20-)30-60 mm long, (5-)15-25(-40) mm wide, broadly elliptical to obovate, with enrolled margins and obtuse to emarginate apex; “winter leaves” slightly to much shorter; leaves yellowish green or tinged purple-reddish, especially near the base. *Scapes* (35-)60-120 mm long, densely glandular. *Calyx* bilabiate, upper lip trilobate (sepals free to the base or fused in the lowermost part), lower lip bilobate, lobes fused for half or more of their length and with their apices obtuse to acute. *Corolla* (13-)16-25(-32) mm long (including spur), bilabiate, upper and lower lip usually spreading at ca. 180° (forming one line), corolla white, usually each corolla lobe with violet-blue tip (but sometimes lobes entirely white), corolla throat and spur yellowish-green, throat sometimes with few reddish-brown stripes; upper two-lobed, lobes divergent, obtuse with emarginate to truncate apex; lower lip deeply trilobed, lobes spreading or slightly overlapping, each with emarginate to truncate apex; spur narrowly cylindrical, (5-)7-13 mm long, straight or slightly

curved downwards. *Seeds* 0.5-0.9 mm long, cylindrical to ellipsoidal, rarely crescent-like, testa reticulate (Shuka *et al.* 2007).

Chromosome number: $2n = 28$ (Mikeladse 1996, Mikeladse & Casper 1997, and Casper & Stimper 2004, 2006 for material from Calabria, Italy; Casper & Stimper 2006 for material from Campania, Italy; Casper & Stimper 2006 and Shuka *et al.* 2007 for material from Albania; Casper *et al.* 2007 and Casper & Stimper 2009 additionally for material from Greece). The repeatedly published karyotypes of $2n = 27$ for this species (for Greece: Strid & Franzén 1981; for Italy: Peruzzi 2004; Peruzzi *et al.* 2007) or ploidy rows ($2n = 16, 24, 32, 48$ for material from Greece: Contandriopoulos & Quezel 1974; Casper 1962) have previously been explained by aneuploidy, but are believed to result from counting errors by Casper & Stimper (2004, 2006, 2009), Casper *et al.* (2007), and Shuka *et al.* (2007).

Distribution: Native to southern Italy (regions Campania and Calabria), the Balkans (Albania, Macedonia), and Greece (regions West Macedonia, Epirus, Thessaly, Central Greece, Peloponnese; Boissier 1875; Casper 1962, 1966; Strid 1991), obviously with an additional outlying finding from Thrakia (Philippos near Drama, Falakro Mountain; sensu Contandriopoulos & Quezel 1974), however which needs confirmation (Strid 1991; no herbarium vouchers of Contandriopoulos or Quezel could be found for this record yet by the author of this article, despite an intensive search); the species is fully absent from the isle of Crete, despite an erroneous mention for that island by Innangi & Izzo, in press). A recent finding of *P. hirtiflora* on Mt. Taygetos, Peloponnese, Greece (<http://www.greekmountainflora.info/Taygetos/Taygetos9.html>) constitutes its southernmost known occurrence (Fig. 1). This species has been naturalized in NW Italy/SE France (two known populations in the Roya Valley; A. Roccia pers. comms.; Fig. 1), Switzerland (one population at Interlaken, canton of Bern, not shown in Fig. 1) and the Czech Republic (one population in the Beskydy Mountains, northern Moravia; Pyšek *et al.* 2012; not shown in Fig. 1).

Habitat: From sea level (e.g. near Vietri Sul Mare, Campania, Italy, where the plants grow on limestone cliffs reached by the spray during heavy sea) to at least 1700 m altitude in Greece (Steiger 1998). High altitude populations of this species hibernate as open, carnivorous leaf rosettes covered by snow and ice. Growing in permanently wet habitats but in a variety of different soils, such as dripping limestone cliffs (Fig. 8), calcareous seepages, margins of streams, wet serpentine rock, peat bogs and even in live *Sphagnum*.

Conservation status: In Italy, there are a few known populations in Campania (Casper 1962, 1970; Pinto *et al.* 2000; Peruzzi *et al.* 2004; Innangi & Izzo, in press), however these are very localized and rare, and thus the species is considered vulnerable in this region (Peruzzi *et al.* 2004). Only a single location is known from Calabria, which is considered critically endangered, as it is under threat by road construction (Peruzzi *et al.* 2004). In the Balkans *P. hirtiflora* var. *hirtiflora* is widespread and common, although sometimes threatened by road constructions (Shuka *et al.* 2007), in Greece several populations also under potential threat by human activities (Steiger 1998).

Notes: The recently proposed *P. lavalvae* (named after Prof. La Valva of Italy; Innangi & Izzo, in press) represents a pale to white flowered population of *P. hirtiflora*. The variation in shape and size of calyx and corolla lobes, as well as the paler color pattern, fall well in the natural range of *P. hirtiflora* var. *hirtiflora*, as it is known between and within populations in the center of the species' distribution in the Balkans and northern Greece. Such pale-flowered plants have been described earlier as *P. hirtiflora* f. *pallida* from Greece and Albania (Casper 1962, 1966). Due to its wide distribution range, *P. hirtiflora* var. *hirtiflora* shows a remarkable variation in coloration, size and shape of its flowers between different populations (Fig. 9), while the plants remain relatively uniform within a population (Steiger 1998).



Figure 8: Left: *Pinguicula hirtiflora* var. *hirtiflora* as a lithophyte on tufa rocks, Poros, Western Makedonia, Greece. Photo by Thassilo Franke. Right: *Pinguicula hirtiflora* var. *hirtiflora* growing on a limestone seepage wall in the Roya valley, France-Italy border, where the species most likely had been introduced to this site.

Cultivation

Cultivation of members of the *P. crystallina*-complex has often been reported to be notoriously difficult (e.g. Adamec & Pasek 2000). In vitro cultivation of most members of this group has proven difficult to impossible (pers. obs.; K. Pasek pers. comms.; P. Harbarth pers. comms.; S. Ippenberger pers. comms.). However, seed propagation in the greenhouse, outdoors or under artificial lights is no problem. If fresh seed is sown, it will readily germinate, and seedlings can be raised to flowering plants within about 1-2 years. Seed or division of old plants is also the only way to propagate these butterworts, as none of the four species will grow from leaf cuttings from my experience (although Slack 1979 reports having made successful leaf cuttings from *P. hirtiflora*; something which I never achieved in any of the different location forms I grow). But some location forms of *P. hirtiflora* var. *hirtiflora* and *P. crystallina* casually will form adventitious plantlets on short lateral outgrowths from the base of the mother plant (very short lateral stolons, not exceeding the overall rosette diameter, so that the adventitious plantlets will usually emerge densely packed to the mother rosette). Further, the rosettes of old healthy specimens of all four species will also regularly divide from the center, usually in spring when in full active growth (but more rarely and to a much lesser degree in *P. hirtiflora* var. *louisii*). So the pots will slowly crowd with several rosettes, even if you started with just a single plant.

My personal experience is that these four species dislike any root disturbance, as most problems and losses arise after repotting. Therefore, the plants are best sown directly into large pots, where they can remain for a long time without removal. Adrian Slack, in his outstanding book on carnivorous plant cultivation, wrote about *P. hirtiflora*: “[...] An easy and worthwhile plant.”, but he also correctly pointed out a crucial point for its cultivation, when stating that “[a]ll the *Pinguicula* hate root disturbance, and should never be repotted while in summer growth. This will almost certainly result in the death of many[...]” (Slack 1979). While I, personally, cannot confirm this for “all the *Pinguicula*” species I grow, this indeed seems to be particularly true for the homophyllous species not forming winter buds (including the *P. crystallina*-complex).

A variety of soil mixes have been used successfully for these species by different growers – my experience is that more clayey substrates will work better than pure peat-sand mixes. I have been using a mix of three parts *Sphagnum* peat, one part grey clay and one part quartzitic sand (for *P.*



Figure 9: The flowers of *P. hirtiflora* var. *hirtiflora* are quite variable in corolla size, shape and coloration. Top: Plants from Aqua Santa, Napoli, Campania, Italy (type locality); Center: from Rossano, Calabria, Italy; Bottom: from Vietri Sul Mare, Campania, Italy.

crystallina from serpentine locations and *P. megaspilaea*) with the addition of another part of calcareous sand or tufa gravel for those growing in alkaline soils (*P. hirtiflora* var. *hirtiflora*, *P. hirtiflora* var. *louisii*, and *P. crystallina* from limestone locations). But all species of this affinity neither seem to be strict calcifuges or calciphiles, and as several *Pinguicula* enthusiasts have told me, all species will happily grow in more or less the very same soil mix.

Cool growing conditions are favoured, and all four taxa grow well for the author in a cool greenhouse, in an area that is shaded from hot summer sun by the aid of shade cloth. Montane location forms of *P. crystallina* and *P. hirtiflora* var. *hirtiflora* will also do well outdoors year round in an



Figure 9 (cont.): Top: from Këlcyra, Albania; Center: from Librazhd, Albania; Bottom: from Mount Olympus, Greece.

alkaline bog garden, in trays or pots, providing protection from bare frost and winter sun (in areas with regular snow cover, growing these species outdoors will cause no problems, otherwise some artificial winter protection by brushwood or cloth is recommended).

Finally, I want to strongly discourage introducing any of these species to natural sites (whether outside or within their natural growing range), as their seed set and vegetative propagation can be enormous in suitable habitats, hence they can constitute a potential invasive threat to native flora. This happened for example in the Roya Valley, along the border of NW Italy and SE France, where naturalized *P. hirtiflora* var. *hirtiflora* of unknown origin has shown to be very invasive, now growing as dense mats on dripping rocks, that suppress the naturally occurring *P. reichenbachiana* at

the same site (A. Roccia pers. comms.; Fig. 8). *Pinguicula hirtiflora* var. *hirtiflora* also has been naturalized at a site in the Czech Republic, where this species had been deliberately planted on a tufa cascade and since forms a spreading population (Pyšek *et al.* 2012).

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PREY CAPTURE PATTERNS IN *NEPENTHES* SPECIES AND NATURAL HYBRIDS –
ARE THE PITCHERS OF HYBRIDS AS EFFECTIVE AT TRAPPING PREY
AS THOSE OF THEIR PARENTS?

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Introduction

The carnivorous pitcher plant genus *Nepenthes* (Nepenthaceae) is thought to comprise more than 120 species, with a geographical range that extends from Madagascar and the Seychelles in the west, through Southeast Asia to New Caledonia in the east (Cheek & Jebb 2001; Chin *et al.* 2014). There are three foci of diversity – Borneo, Sumatra, and the Philippines – which account for more than 75% of all known species (Moran *et al.* 2013). The pitchers of *Nepenthes* have three main components – the pitcher cup, the peristome (a collar-like band of lignified tissue that lines the pitcher mouth), and the lid (Fig. 1A-G). In most species, the lid is broad and flat and overhangs the mouth (Fig. 1B-D), but in some specialized species it is small and oriented away from the mouth (Fig. 1A,E). The inner walls of the pitcher cup may be divided into two discrete zones – a lower “digestive” zone in which the pitcher walls lack a waxy cuticle and are lined with digestive glands; and an upper “conductive” zone, which lacks digestive glands but is covered by a complex array of wax crystals (Juniper *et al.* 1989; Bonhomme *et al.* 2011). Insects that make their way onto the conductive surface often lose their footing and fall into the digestive zone, which contains a viscoelastic fluid that facilitates the retention and drowning of prey.

Ever since *Nepenthes* were first encountered by Europeans, scientists have attempted to determine how the various components of the trap operate (Lloyd 1942; Phillipps & Lamb 1996). However, it was not until relatively recently that the structure and function of the peristome were properly elucidated, a discovery that has had a profound influence on subsequent research (Bohn & Federle 2003). It has been demonstrated that the peristome is a highly effective trapping surface when it is moist, but not when it is dry, due to its anisotropic, wettable surface microstructure (Bauer *et al.* 2008). By contrast, the effectiveness of the waxy zone is independent of moisture levels (Bauer *et al.* 2012a; Moran *et al.* 2013). An enlarged peristome is thought to have lower construction costs compared to a well-developed waxy zone (Poorter & De Jong 1997; Riedel 2007), so in perhumid environments (i.e., those that experience high levels of rainfall throughout the year and lack distinct dry seasons), traps with reduced waxy zones and expanded peristomes are likely to be the most efficient at trapping prey (Bauer *et al.* 2012a; Moran *et al.* 2013). However, in seasonal environments, a well-developed waxy zone and narrow peristome will be effective even if the weather is relatively dry for short periods. For this reason, the species with the largest, most spectacular pitchers are effectively confined to equatorial habitats in Southeast Asia that experience a perhumid climate, whereas the narrow peristome/extensive waxy zone trap format is found throughout the geographical range of the genus (Moran *et al.* 2013).

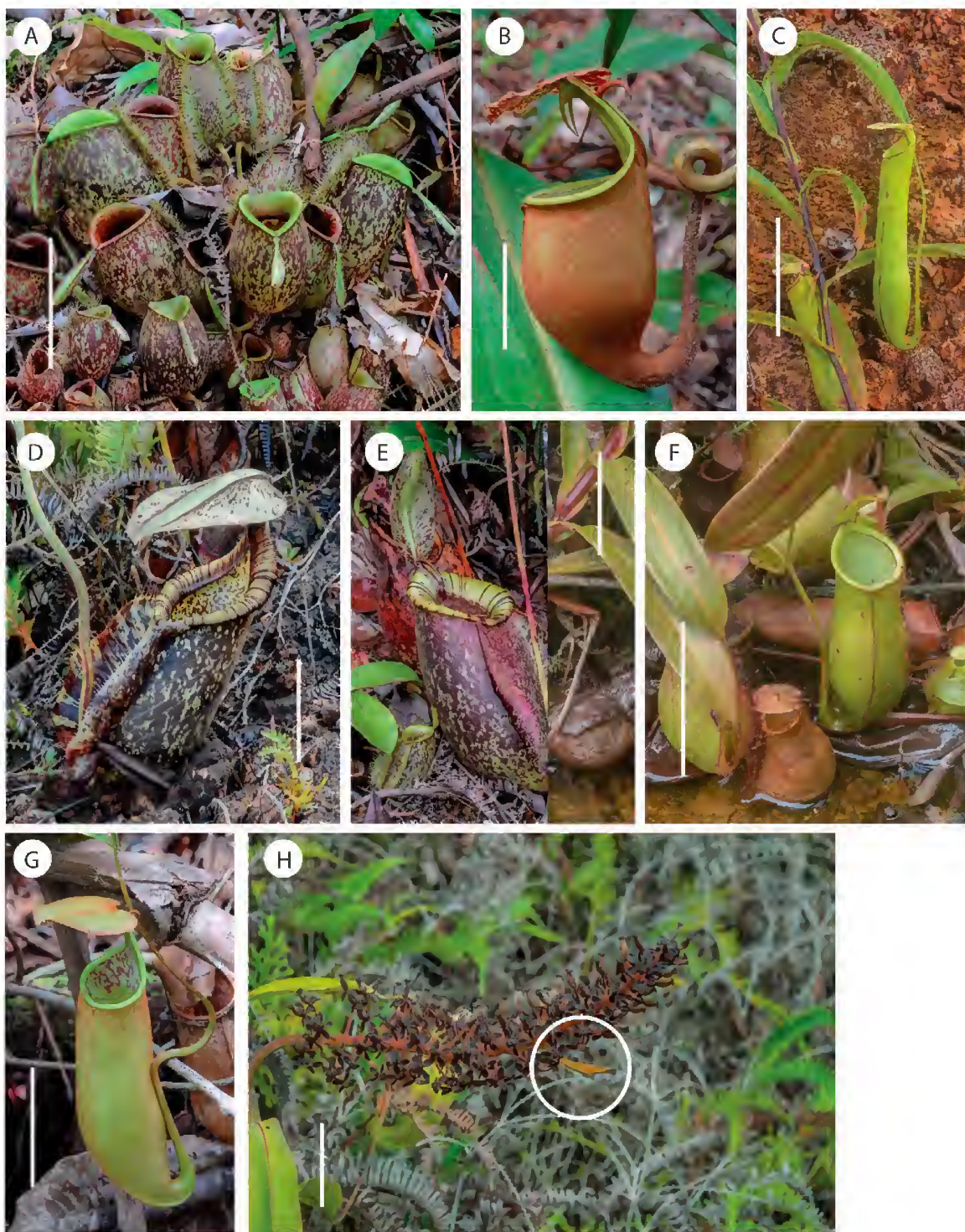


Figure 1: Representative images of the seven *Nepenthes* taxa included in this study. A. Terrestrial rosette pitchers of *Nepenthes ampullaria*, Mersing. B. Aerial pitcher of *Nepenthes bicalcarata*, Serian. C. Aerial pitcher of *Nepenthes gracilis*, Mersing. D. Terrestrial pitcher of *Nepenthes rafflesiana*, Mersing. E. Terrestrial pitcher of *Nepenthes* \times *hookeriana*, Mersing. F. Intermediate pitcher of *Nepenthes* \times *trichocarpa*, Matang. G. Aerial pitcher of *Nepenthes* \times *cantleyi*, Serian. H. Female inflorescence of *N. ampullaria* \times *N. gracilis*, Matang, Sarawak. Note the single fruit (circled). Scale bar in all images = 5 cm.

Open, moist sunny sites with poor soils are thought to provide optimal conditions for the carnivorous syndrome in plants (Givnish *et al.* 1984). Several common *Nepenthes* species, such as *N. gracilis*, *N. rafflesiana*, and *N. mirabilis*, frequently colonise sandy, nutrient-deficient substrates where the vegetation has been heavily disturbed, and, as these are among the commonest species in the Sunda region, this has led to a general impression among workers that the genus as a whole tends to prefer disturbed habitats (Phillipps & Lamb 1996; Clarke 1997). In fact, apart from a small number of species that are well-adapted to highly disturbed habitats, the majority of species grow in undisturbed vegetation that occurs on nutrient-deficient soils, such as tropical heath, peat swamp and upper montane forests, and/or grow as epiphytes in intact forest canopies (Clarke 2001; Chin *et al.* 2014). The vegetation in disturbed sites is often similar in structure to these formations (i.e., having a low, open canopy, patches of bare ground and an abundance of small shrubs), but as the majority of biologists who work with *Nepenthes* have tended to focus their efforts on a few widespread, common *Nepenthes* species that grow in disturbed sites, a skewed perception of what represents a “typical habitat for *Nepenthes*” has arisen.

Hybrids and hybridization in *Nepenthes*

This misconception has given rise to another: that *Nepenthes* frequently produce natural hybrids in the wild. *Nepenthes* are dioecious and produce fertile hybrids, and many putative natural hybrids have been reported (Clarke 1997, 2001). In suitable habitats in Borneo, Sumatra and the southern Philippines, it is not unusual to encounter two or more *Nepenthes* species growing in mixed populations at the same locality. If the vegetation at such sites has been recently disturbed (as is the case in most field sites used in previous research), natural hybrids may be common, but in undisturbed habitats, natural hybrids are actually very rare. The most likely explanation for this is that the majority of co-occurring *Nepenthes* species in undisturbed sites appear to be reproductively isolated due to staggered flowering times (C. Clarke, pers. observ.). However, disturbances to the plants’ habitats can disrupt the normal flowering seasons, resulting in overlap between species that are normally temporally isolated with regards to their phenology. When this happens, cross-pollination may occur, giving rise to hybrid progeny. There is little documented evidence to support these patterns at present – again; this is because few biologists have conducted detailed surveys of *Nepenthes* phenology in undisturbed habitats. One (qualitative) example involves mixed, co-occurring populations of *N. veitchii*, *N. hurrelliana* and *N. chaniana* on Mt. Batu Buli in northern Sarawak. In 1988, one of us (CC) visited this mountain which, at the time, supported pristine montane forest. The three species of *Nepenthes* mentioned above were common in this forest, but only one or two putative hybrids were found among a multi-species population that numbered in the thousands of plants. On a subsequent visit to the same area in 2007, a few years after the site had been disturbed by the construction of a logging road (and subsequent removal of many of the canopy trees in the area), the overall number of plants at the site was much reduced, but putative hybrids involving all three species were common, possibly accounting for as much as 10% of the *Nepenthes* plants that grew along the old logging road. At similar disturbed sites in the lowlands of northwestern Borneo, natural hybrids were observed by CC to be common in the late 1980’s, but once the disturbances in this area ceased and the forest recovered (c. 2012) most of the hybrids had died out, whereas the parent species remained.

Thus, based on the meager, qualitative evidence available, it seems that most *Nepenthes* species that grow in undisturbed habitats rarely produce natural hybrids, but when significant habitat disturbances occur, hybridization is more common. Furthermore, in the absence of ongoing habitat disturbances, hybrid plants appear to be unlikely to persist and form independent, stabilized populations of their own. Given that the few phylogenetic studies of *Nepenthes* based on nucleic acids (e.g.,

Meimberg & Heubl 2006; J. Mullins, unpublished) propose that introgression is widespread in the genus, it seems incongruous that hybrid plants appear to be “unsuccessful” at persisting in the wild.

There are two primary mechanisms by which natural hybrids can confer evolutionary advantage, either to the genus as a whole, and/or to one or both of their parent species. The first is that the hybrid plants themselves are more competitive (or have greater evolutionary fitness) than the parents, and are capable of reproducing effectively among themselves. Over time, the hybrid plants increase in number and become reproductively isolated from the parent species, eventually becoming established as a new species (Clarke 1997). Alternatively, the hybrid plants may fail to compete effectively, and/or become independent of the parents, but they may cross back (this is called introgression) with one or more parent species, thereby increasing the size of the gene pool of the parent species and enhancing its ability to respond to changes in selective pressures that might arise, say, from processes such as climate change or habitat disturbance (Clarke 2001).

Prey capture in *Nepenthes* and the characteristics of *Nepenthes* hybrids

It is common for the pitchers of natural *Nepenthes* hybrids to appear “intermediate” between those of their parent species (Clarke 1997). For instance, if one species that produces narrow, tubular pitchers with well-developed waxy zones (e.g., *N. gracilis* (Fig. 1C)) is crossed with another that produces squat, ovoid ones that lack a waxy zone, but have a large, expanded peristome (e.g., *N. ampullaria* (Fig. 1A)), the pitchers of the offspring tend to be broader and more ovoid than those of *N. gracilis*, but taller and narrower than those of *N. ampullaria*, with a small waxy zone and a slightly expanded peristome (e.g., *N. ampullaria* × *N. gracilis* (= *N. × trichocarpa*), Fig. 1F). Clarke (1997) examined the metazoan invertebrate community from pitchers of *N. bicalcarata*, *N. gracilis* and the natural hybrid, *N. bicalcarata* × *N. gracilis* (= *N. × cantleyi*) (Fig. 1B, C, and G), and found that even the structure and composition of the animal community of the hybrid was intermediate between those of its parent species. Given that both *N. bicalcarata* and *N. gracilis* have been shown to have specialized trap function (Clarke & Kitching 1995; Bauer *et al.* 2012b), what are the implications of “being intermediate” for prey capture by hybrid *Nepenthes*?

Recent research into nutrient acquisition in *Nepenthes* has demonstrated that species with highly specialized pitcher structures tend to have highly specialized nutrient sequestration strategies. In other words, species that target a particular type of prey appear to produce pitchers with modifications that make them particularly attractive to that prey (Chin *et al.* 2010; Chin *et al.* 2014). One example is *N. albomarginata*, which deploys a band of dense white hairs beneath the peristome that attracts termites, which feed on the hairs and are then trapped in large numbers by the pitchers (Moran *et al.* 2001; Merbach *et al.* 2002). Furthermore, *N. ampullaria* traps falling leaf litter in addition to insects by producing a “carpet” of pitchers at ground level (Fig. 1A), that have small, reflexed lids that do not cover the pitcher mouths. This allows falling detritus to enter the pitchers freely, and such inputs account for a significant proportion of foliar N in this species (Moran *et al.* 2003). More recently, the outsized, highly modified pitchers of *N. lowii*, *N. rajah* and *N. macrophylla* have been shown to attract mountain tree shrews (*Tupaia montana*), which defecate into the pitchers in return for a reward of nutritious nectar provided by glands on the pitchers’ lids (Clarke *et al.* 2009; Chin *et al.* 2010).

Chin *et al.* (2014) posed the question: if gross modifications to *Nepenthes* pitcher characteristics facilitate specialized nutrient sequestration strategies, what about species that lack these? Do species that lack obvious pitcher specializations behave as generalist predators, trapping whatever insects happen to encounter the pitchers, or could they also be specialists, targeting specific types of arthropods over others, using subtle specializations to trap structure that are not obvious to human observers? To investigate this question, they studied prey capture patterns in eight *Nepenthes*

species from three localities in Borneo. Of these, four species produce pitchers with unique characteristics and specialized function (*N. ampullaria*, *N. bicalcarata*, *N. lowii*, *N. macrophylla*), whereas the remainder (*N. gracilis*, *N. mirabilis*, *N. rafflesiana*, *N. tentaculata*) do not. Their findings demonstrated when prey was identified to the taxonomic level of Order, the species with specialized pitchers could be easily distinguished through simple quantitative analyses, but that differences in prey capture strategies among the “non-specialists” were subtle and not readily explained. In all lowland species examined to date, ants (Formicidae) were the numerically dominant prey taxon, and it was only when the ants were identified to the level of genus and/or species that significant interspecific differences in prey capture strategies among these *Nepenthes* became evident.

Chin *et al.* (2014) concluded that it is possible that co-occurring *Nepenthes* species that trap mostly ants, using pitchers that lack gross morphological modifications, could target different combinations of ant taxa (presumably using subtle, or less obvious adaptations), thereby avoiding competition for prey. However, they also noted that the evidence they obtained to support this conclusion was not compelling and there may be alternative explanations for the patterns they found. However, if co-occurring *Nepenthes* do target different combinations of arthropod taxa, then comparison of their prey spectra to those of their natural hybrids could provide further insights into interspecific differences in prey capture patterns, as well as the effectiveness of hybrid pitchers in trapping prey. Such insights might also shed light on the ecological fitness of natural hybrids (or lack thereof).

Do hybrid *Nepenthes* trap prey as effectively as their parent species?

In species that have similar pitcher structure, such as *N. gracilis* and *N. mirabilis*, the fact that the pitchers of most natural hybrids are intermediate in appearance between those of their parents does not result in any obvious loss of pitcher function. However, in hybrids that involve one or more highly specialized parent species (such as *N. ampullaria* or *N. bicalcarata*), specialized pitcher characters may be lost, greatly reduced or rendered ineffective. Returning to the example of *N. bicalcarata* × *N. gracilis*; the distinctive thorns of *N. bicalcarata* pitchers are reduced to small bumps in the hybrid (Fig. 1G), so that any function they may serve in *N. bicalcarata* pitchers is not inherited. In such cases, do hybrids such as *N. bicalcarata* × *N. gracilis* inherit “the worst of both worlds” – losing the specializations of their parents, and lacking the ability to target any arthropod prey groups effectively? If so, it is possible that hybrids such as this would have difficulty obtaining the nutrients required to reach maturity and flower and set viable seed. And, if so, this could go some way to explaining their inability to persist in the wild.

At present, we can do little but postulate about the evolutionary and ecological fitness of *Nepenthes* hybrids, as no detailed ecological observations or experiments have been performed. In this study, we sought to establish a platform upon which further research projects might be based, by comparing prey capture patterns of three natural *Nepenthes* hybrids and their co-occurring parent species at three locations in Malaysia. We compared measures of several physical attributes of the pitchers (to find out whether hybrid pitchers are indeed intermediate in structure compared to those of their parents), and analyzed patterns in prey capture to address the hypothesis that hybrid *Nepenthes* plants are less effective at trapping prey than their specialized parent species.

Materials and Methods

Nepenthes taxa

Seven *Nepenthes* taxa, including four species and three natural hybrids, were included in the survey. The selection criterion for species and hybrids was straightforward – in order to compare

patterns of arthropod prey capture using quantitative analyses, it was necessary to select hybrid taxa that grew in mixed populations with both of their parent species at the same site, and which were sufficiently common to satisfy the most fundamental assumptions of the analytical methods used. These constraints preclude all but the most commonly occurring natural hybrids and within Malaysia, we were only able to identify three hybrids and localities that were suitable for this type of study. These were: *N. ampullaria* × *N. gracilis* (= *N. × trichocarpa*), *N. bicalcarata* × *N. gracilis* (= *N. × cantleyi*), and *N. ampullaria* × *N. rafflesiana* (= *N. × hookeriana*).

Study sites

The study was performed at three geographically isolated sites in Malaysia: one near the town of Mersing in Johor (on the Malay Peninsula) and the other two near the city of Kuching in Sarawak on the island of Borneo. The selection criteria for study sites were based on two factors: accessibility and suitability for running extended field experiments, and abundance of two species of *Nepenthes* and their putative natural hybrid growing together (and hence, exposed to the same pool of potential prey organisms) at the site. The first site (called “Serian” in this study), was located to the SE of Kuching, at an altitude of 37 m above sea level (asl). Since this study was performed in 2010, the vegetation at this site has been destroyed, but when our experiments were conducted, it supported fragments of several types of vegetation, ranging from open, bare sandy ground to intact peat swamp forest. Originally, the area supported a mosaic of peat swamp forest and tropical heath forest (*kerangas*), much of which has now been cleared. Five species of *Nepenthes* occurred at this site: *N. ampullaria*, *N. bicalcarata*, *N. gracilis*, *N. mirabilis* and *N. rafflesiana*. The species and hybrid that we surveyed at this site were *N. bicalcarata*, *N. gracilis* and *N. bicalcarata* × *N. gracilis*. Plants of *N. bicalcarata* were virtually confined to the intact peat swamp forest fragments, whereas *N. gracilis* grew at the margins of the intact forest and in open areas. Hybrids of these species grew both under the intact forest canopy and in open areas. The second site (referred to as “Matang”), was located on a roadside near the village of Matang, to the north of Kuching. Three species of *Nepenthes*, *N. ampullaria*, *N. gracilis* and *N. rafflesiana*, grow in mixed populations on open, exposed embankments on both sides of the road. The vegetation is sparse *adinandra belukar* (an anthropogenic shrubland formation that resembles degraded *kerangas* (Sim *et al.* 1992)), which is characterized by patches of bare ground, interspersed with patches of resam fern (*Dicranopteris linearis*) and small shrubs. This site was chosen because it also supports large numbers of the natural hybrid, *N. ampullaria* × *N. gracilis*.

The third site (called “Mersing”) was in the Malaysian state of Johor, near the town of Mersing, on the west coast of the Malay Peninsula. Like the Matang site in Sarawak, the plants grew in clearings by the sides of roads, in sparse *adinandra belukar* shrubland. Three species of *Nepenthes* (*N. ampullaria*, *N. gracilis*, and *Nepenthes rafflesiana*) occurred at this site, along with plants of two natural hybrids: *N. ampullaria* × *N. gracilis* and *N. ampullaria* × *N. rafflesiana*. Figure 1 presents photographs of all seven *Nepenthes* taxa included in the study.

Sampling methods

Pitchers were selected for study on the basis of their age and condition. Very old and very young pitchers, or those that displayed obvious signs of damage, may not be fully functional and hence were excluded. Maximum sample sizes were imposed by logistical constraints. We had sufficient human resources to study 10-30 pitchers of each species and hybrid at each site. The final numbers sampled were the number of pitchers that were still intact and operational at the end of the experiment (Table 1). All pitchers sampled were from separate plants. Where pronounced intra-specific pitcher dimorphism occurred, we attempted to sample equal numbers of both pitcher types, to ac-

Table 1. Summary statistics for prey capture by the seven *Nepenthes* taxa studied. Values are Means $1 \pm$ S.D.

<i>Nepenthes</i> species and location						
	Serian, Sarawak				Matang, Sarawak	
Prey inputs	<i>bicalcarata</i>	<i>gracilis</i>	\times <i>cantleyi</i>	<i>ampullaria</i>	<i>gracilis</i>	\times <i>trichocarpa</i>
Number of pitchers sampled	28	25	30	33	32	27
Mean number of individual prey items	38.607 \pm 10.532	1.440 \pm 0.664	5.867 \pm 1.394	1.455 \pm 0.348	3.156 \pm 0.767	3.778 \pm 1.743
Mean number of prey taxa	2.464 \pm 0.311	0.600 \pm 0.173	0.967 \pm 0.153	0.667 \pm 0.128	1.156 \pm 0.156	0.852 \pm 0.157
Arthropod Prey Taxa						
Coleoptera	0.321 \pm 0.155	0.040 \pm 0.040	0.067 \pm 0.046	0.091 \pm 0.091	0.156 \pm 0.079	0.074 \pm 0.051
Dictyoptera	0	0	0	0.030 \pm 0.030	0	0
Hemiptera	0.107 \pm 0.060	0	0.067 \pm 0.046	0.030 \pm 0.030	0	0.037 \pm 0.037
Lepidoptera	0.107 \pm 0.060	0	0	0	0.031 \pm 0.031	0
Diptera	0.250 \pm 0.083	0.160 \pm 0.075	0.133 \pm 0.078	0.152 \pm 0.063	0.594 \pm 0.190	0.259 \pm 0.137
Orthoptera	0.071 \pm 0.050	0	0.033 \pm 0.033	0	0.031 \pm 0.031	0
Formicidae	26.214 \pm 7.621	1.040 \pm 0.644	4.533 \pm 1.245	1.152 \pm 0.335	2.281 \pm 0.760	3.407 \pm 1.732
Arachnida	0.250 \pm 0.098	0.040 \pm 0.040	0	0	0.031 \pm 0.031	0
Termitoidae	10.821 \pm 3.939	0.120 \pm 0.120	1.033 \pm 0.641	0	0	0
Acarina	0.429 \pm 0.181	0	0	0	0	0
Mersing, Johor						
Prey inputs	<i>ampullaria</i>	<i>gracilis</i>	<i>rafflesiana</i>	\times <i>hookeriana</i>	\times <i>trichocarpa</i>	
Number of pitchers sampled	29	12	32	36	11	
Mean number of individual prey items	2.172 \pm 0.632	12.667 \pm 10.773	8.000 \pm 1.468	1.722 \pm 0.427	1.364 \pm 0.621	
Mean number of prey taxa	0.759 \pm 0.146	0.833 \pm 0.167	1.688 \pm 0.278	0.611 \pm 0.121	0.818 \pm 0.310	
Arthropod Prey Taxa						
Coleoptera	0.069 \pm 0.048	0	0.406 \pm 0.173	0	0	
Dictyoptera	0	0	0.156 \pm 0.091	0	0	
Hemiptera	0	0	0.031 \pm 0.031	0	0	
Lepidoptera	0	0	0.125 \pm 0.087	0	0	
Diptera	0	0.083 \pm 0.083	0.250 \pm 0.090	0	0.091 \pm 0.095	
Orthoptera	0	0	0	0	0.091 \pm 0.095	
Formicidae	1.828 \pm 0.594	12.583 \pm 10.778	5.656 \pm 1.338	1.222 \pm 0.359	1.091 \pm 0.435	
Arachnida	0.034 \pm 0.034	0	0.156 \pm 0.091	0	0.091 \pm 0.095	
Termitoidae	0.207 \pm 0.144	0	1.125 \pm 0.575	0.222 \pm 0.127	0	
Acarina	0.304 \pm 0.304	0	0.063 \pm 0.043	0.222 \pm 0.155	0	

count for potential effects of dimorphism. If more than 25 suitable pitchers of a given species could be found at a site, 25-35 of these were randomly selected for study. If less than 20 suitable pitchers were available, all of them were tagged and used.

Several physical characteristics of the pitchers were measured, including: The width and length of the pitcher orifice at their widest points, the width and length of the pitcher lid at their widest points, the width of the peristome at the side of the pitcher mouth, the pitcher height at the rear (measured from the base of the spur to the lowermost point of the pitcher), the pitcher height at the front (measured from the top of the peristome to the lowest point of the pitcher), the lengths of the wax zones at the front and rear of the pitcher, and the pitcher capacity. These characteristics may or may not have important roles in (targeted) prey capture strategies – they were chosen purely to assist in the description of overall pitcher structure and their potential roles in pitcher function were not investigated.

We used the method of Moran (1996) to survey arthropod prey capture in *Nepenthes* pitchers. This involves clearing the pitchers of their existing contents and “re-setting” them, so that they capture prey for a fixed, uniform period, thereby enabling direct comparison of capture rates among all pitchers. Pitchers were emptied of their contents and rinsed with distilled water. The contents were then passed through filter paper to remove all macroscopic detritus. The volume of the fluid was then measured to the nearest ml and returned to the pitcher, whereas the detritus was discarded. Pitchers were then left for 14 days to capture prey. This time interval was chosen as it allows the longest possible period for pitchers to trap prey without providing the invertebrate fauna sufficient time to re-colonize the pitchers and degrade the prey to the point where identification becomes difficult. At the conclusion of the experiment, the contents of the pitchers were poured into a 250 ml beaker and the inner surfaces of the pitchers were thoroughly rinsed with distilled water to remove all of the contents. The fluid was filtered once more, but this time the contents were retained and preserved in 70% ethanol for sorting and identification, whereas the filtrate was returned to the pitchers. Remains of captured prey were sorted and identified to the level of Order. Ants were identified to the subordinal rank (Formicidae) to distinguish them from other Hymenoptera, such as bees and wasps.

Data Analysis

All summary statistics are presented as means \pm 1 SD or SE (depending on the analysis). Descriptive statistics and principal components analyses were calculated and analysed using Minitab v. 16. All decisions about hypotheses were made against a critical value of $\alpha = 0.05$.

Quantitative analysis of prey capture patterns were conducted at the Ordinal level for all main arthropod taxa, except for ants (Formicidae) due to their importance as prey in lowland pitchers.

Prey capture patterns were depicted using a series of “star plots”, which display proportional abundances of prey taxa for each *Nepenthes* species, in which each taxon is represented by a “wedge” in a circular chart Ellison and Gotelli (2009). The size of the wedge was scaled in proportion to the amount of total prey that was accounted for by any given taxon. All taxa listed in the key were trapped by pitchers; taxa that are not visible in any particular plots were either not captured at all by that particular *Nepenthes* taxon, or were not captured in sufficient numbers to be resolved. The minimum level of resolution in the star plots was four percent of total prey caught. Each star chart contains three concentric circles (colored red). In order of increasing size, these denote prey composition values of 12.5, 25, and 50%, respectively.

To determine whether different *Nepenthes* species specialize on particular prey taxa, we compared estimates of Hurlburt’s *PIE*, using single-factor ANOVA and Tukey’s pairwise comparisons. To test for evidence of niche segregation, we performed null-model analysis using the ‘RA-3’ algo-

rithm within the EcoSim software package to quantify niche overlap using Pianka's index of overlap in resource use (Hurlburt 1971; Pianka 1973; Gotelli & Graves 1996; Gotelli & Entsminger 2007). These methods are outlined in greater detail in Ellison & Gotelli (2009) and Chin *et al.* (2014).

Results

Principal component analyses of the pitcher characteristics demonstrated that, at least in terms of gross pitcher morphology, the pitchers of the hybrid *Nepenthes* are intermediate between those of their parent species (Fig. 2A-C). At Matang, the first two principal components described 79.0% of the variation in the data, while at Serian the corresponding value was 85.5% and at Mersing it was 77.2%. The descriptive statistics for the three PCAs are presented in Table 2. Investigation of the loadings for each PC was inconclusive, with no consistent patterns found across the three study sites. This is not surprising – the parent species differ substantially in pitcher structure, so every pitcher component that was measured contributes significantly to the levels of variation detected, meaning that the likelihood of detecting any consistent trends among pitcher characteristics within and among study sites was low.

In the pitchers of all taxa, ants (Formicidae) were the dominant prey type (Table 1, Figs. 3 & 4). This pattern is typical of the vast majority of lowland *Nepenthes* species studied to date (Moran 1996; Adam 1997; Chin *et al.* 2014; Clarke *et al.* 2014), and appears to indicate that ants are the most important type of prey for these plants. The second most abundant prey taxon varied among sites: Diptera at Matang (Fig. 3a), Termitoidae at Serian (where the *Nepenthes* plants grow in and around closed forest) (Fig. 3b), and a variety of taxa at Mersing (Fig. 4). The prey spectra of the hybrids more or less matched their parent species, indicating that all pitchers within a site were exploiting the same prey, and trapping prey taxa (when resolved ordinal rank) in similar proportions, regardless of pitcher characteristics.

Table 3 presents the results of a series of t-tests performed to compare prey capture between *Nepenthes* species (all species at a study site being treated as a single entity) and hybrids (ditto). At Serian and Mersing, the pitchers of *Nepenthes* species caught greater numbers and diversity of prey, but there were no differences at Matang. These results indicate that pitchers in three of the four hybrid swarms examined were less effective at trapping prey than their parent species. In all but two cases (*N. bicalcarata* at Serian and *N. gracilis* in Johor), prey capture rates per pitcher were very low (Table 1), particularly when compared with the findings of Chin *et al.* (2014). This result is intriguing, as the study undertaken by Chin *et al.* (2014) was run at the same time as the present one using the same sampling methods, yet the prey capture rates appear to be somewhat lower.

There were no significant differences in levels of specialization towards prey among co-occurring *Nepenthes* species and hybrids at Mersing ($F_{4,68} = 2.13$, $P = 0.087$) or Matang ($F_{2,57} = 0.49$, $P = 0.616$), but at Serian there was a significantly higher mean value for PIE in *N. bicalcarata* compared to *N. ampullaria* \times *N. bicalcarata* ($F_{2,52} = 6.00$; $P = 0.005$). The results of the null-model analysis show no evidence for niche segregation at any of the three study site/hybrid-species combinations (Table 4). These results concur with those obtained by Chin *et al.* (2014) for niche overlap analysis for prey that is resolved only to the ordinal rank.

Discussion

Summary of our findings and limitations to the experimental design

The results of our analyses demonstrate that, in terms of the morphological characteristics that we measured, the properties of hybrid *Nepenthes* pitchers do lie somewhere between those of their

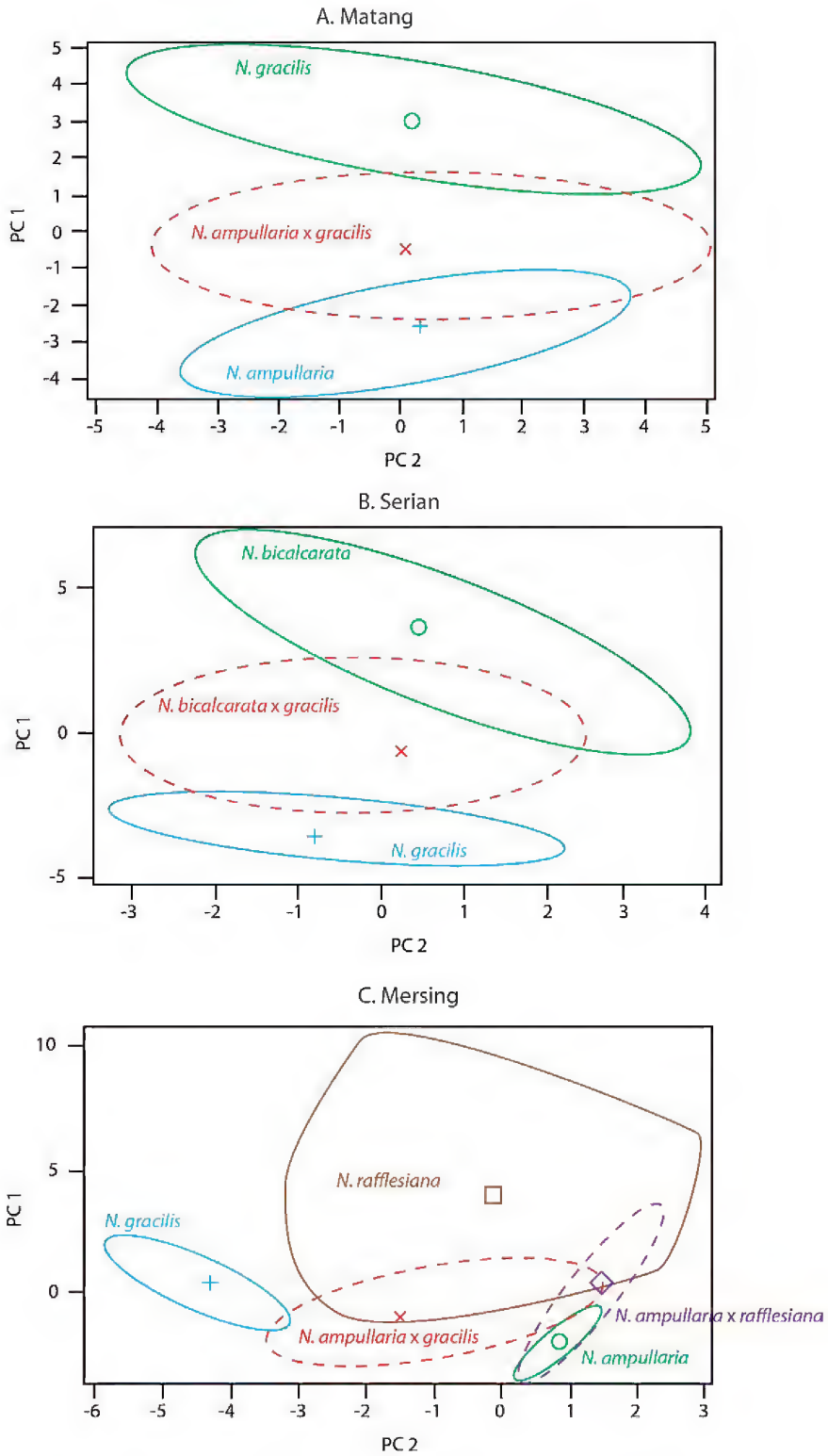


Figure 2: Plots of scores for the first two principal components obtained from PCA of the morphological characteristics of the pitchers of the various *Nepenthes* taxa studied. A. Results for pitchers at Matang. B. Results for pitchers at Serian. C. Results for pitchers at Mersing. For each taxon, within each plot, the mean for PCs 1 & 2 is denoted by a symbol, while the range of values of scores for individual pitchers is described by an ellipse (or polygon, in the case of *N. rafflesiana*). The symbols and ellipses are color-coded to clearly distinguish each taxon.

Table 2. Eigenvectors and contributions of the first three principal components and scores for variables in the principal component analyses for pitcher characteristics at each site.									
Site	Matang			Serian			Mersing		
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
Eigenvalue	4.876	3.020	0.686	6.739	1.814	0.531	5.751	1.965	0.611
Proportional contribution	0.488	0.302	0.069	0.674	0.181	0.053	0.575	0.196	0.061
Cumulative contribution	0.488	0.790	0.858	0.674	0.855	0.908	0.575	0.772	0.833
Variable (pitcher characteristic)									
Mouth length	0.153	-0.457	0.337	0.368	-0.018	0.043	-0.375	-0.045	-0.008
Mouth width	-0.005	-0.467	0.310	0.350	-0.139	0.001	-0.277	0.016	0.940
Lid length	0.314	-0.343	-0.289	0.282	0.029	-0.929	-0.385	-0.127	-0.075
Lid width	-0.335	-0.114	0.668	0.361	-0.043	0.155	-0.377	0.001	-0.040
Peristome width	0.399	-0.109	0.014	0.356	0.150	0.114	-0.285	-0.345	-0.064
Pitcher height at rear	-0.296	-0.389	-0.257	0.315	-0.357	-0.030	-0.393	0.029	-0.145
Pitcher height at front	-0.223	-0.413	-0.418	0.156	-0.646	0.118	-0.333	0.107	-0.235
Wax zone length at pitcher front	-0.423	-0.103	-0.123	-0.283	-0.472	-0.044	-0.079	0.680	-0.044
Wax zone length at pitcher rear	-0.428	-0.070	-0.058	-0.294	-0.420	-0.198	-0.154	0.614	-0.008
Capacity	0.328	-0.304	0.038	0.340	-0.102	0.203	-0.340	-0.106	-0.162

parents, and that at two of the three study sites we used, hybrid pitchers caught lower numbers and fewer types of prey (Fig. 2, Table 1). We did not detect evidence of niche segregation with regards to prey capture among any species-hybrid combinations (within study sites), and apart from *N. bicalcarata* at Serian, we did not detect any differences in specialization with regards to prey capture among *Nepenthes* species and hybrids.

There are some important limitations to the quality of the data we obtained, and this impacted upon the results of the subsequent analyses. First, we lacked the expertise to identify the ant species below the rank of suborder (Formicidae). As noted by Chin *et al.* (2014), evidence for niche segregation was only detected when ants were resolved to the rank of genus or species, so although we did not detect evidence for niche segregation in this study, that does not mean that it could not exist. We made no attempt to determine whether the prey spectra of any of the *Nepenthes* taxa we surveyed indicated specialization by the plants towards specific prey type(s) – the objective was purely to compare prey capture patterns among species and hybrids that are exposed to the same arthropod communities. Chin *et al.* (2014) provided an exhaustive review of the limitations to their experimental design, and those limitations also apply to this study. For the sake of brevity, that discussion is not repeated here.

Although the morphological parameters we measured indicate that hybrid pitchers are “the average of those of their parents”, this does not mean that all other aspects of hybrid pitchers necessarily conform to this pattern. In particular, non-physical characteristics of pitchers, such as visual and

Table 3. Comparisons of prey capture patterns between pitchers of <i>Nepenthes</i> species and hybrids at each of the three study sites. * Denotes a significant difference at $P = 0.05$.						
Comparison	Taxa	Number of pitchers	Mean	S.E.	$t_{\text{d.f.}}$	P
Matang						
Number of prey types caught	Species	65	0.91	0.10	$t_{50} = 0.30$	0.770
	Hybrids	27	0.85	0.16		
Number of prey items caught	Species	65	2.29	0.43	$t_{29} = 0.83$	0.410
	Hybrids	27	3.78	1.70		
Serian						
Number of prey types caught	Species	53	1.58	0.22	$t_{80} = 2.28$	0.025*
	Hybrids	30	0.85	0.16		
Number of prey items caught	Species	53	44.40	6.10	$t_{57} = 2.50$	0.018*
	Hybrids	30	7.74	1.40		
Mersing						
Number of prey types caught	Species	74	1.18	0.14	$t_{117} = 2.81$	0.006*
	Hybrids	46	0.80	0.12		
Number of prey items caught	Species	74	6.40	1.90	$t_{78} = 2.50$	0.014*
	Hybrids	46	1.63	0.36		

Table 4. Summary of null model analysis of niche overlap in prey utilization. "Observed" is the observed average pair-wise niche overlap. "Expected" is the mean value of average pairwise niche overlap in 10000 randomizations of the resource utilization data. The P value is the upper tail probability of finding the observed pattern if the data were drawn from the null distribution.				
Site	Number of taxa	Observed	Expected	P
Mersing	5	0.98034	0.15049	< 0.001
Serian	3	0.97416	0.17679	< 0.001
Matang	4	0.99261	0.14741	< 0.001

olfactory cues, require detailed investigation to determine how they operate in the parent species, and then how or whether they also operate in hybrids. However, for the purposes of this discussion, we assume that our findings are representative with regards to the physical structure of the pitchers, and we follow the argument of Chin *et al.* (2014) that the pitchers of *N. bicalcarata* and *N. ampullaria* are specialized with regards to nutrient sequestration strategies (Clarke & Kitching 1995; Moran *et al.* 2003).

Implications of our findings

Of the three hybrids examined in this study, *N. bicalcarata* × *N. gracilis* provides the clearest example of the way in which the specialized characteristics of the pitchers of one parent species (*N. bicalcarata*) are effectively neutralized in hybrid progeny. Four distinctive features of *N. bicalcarata*

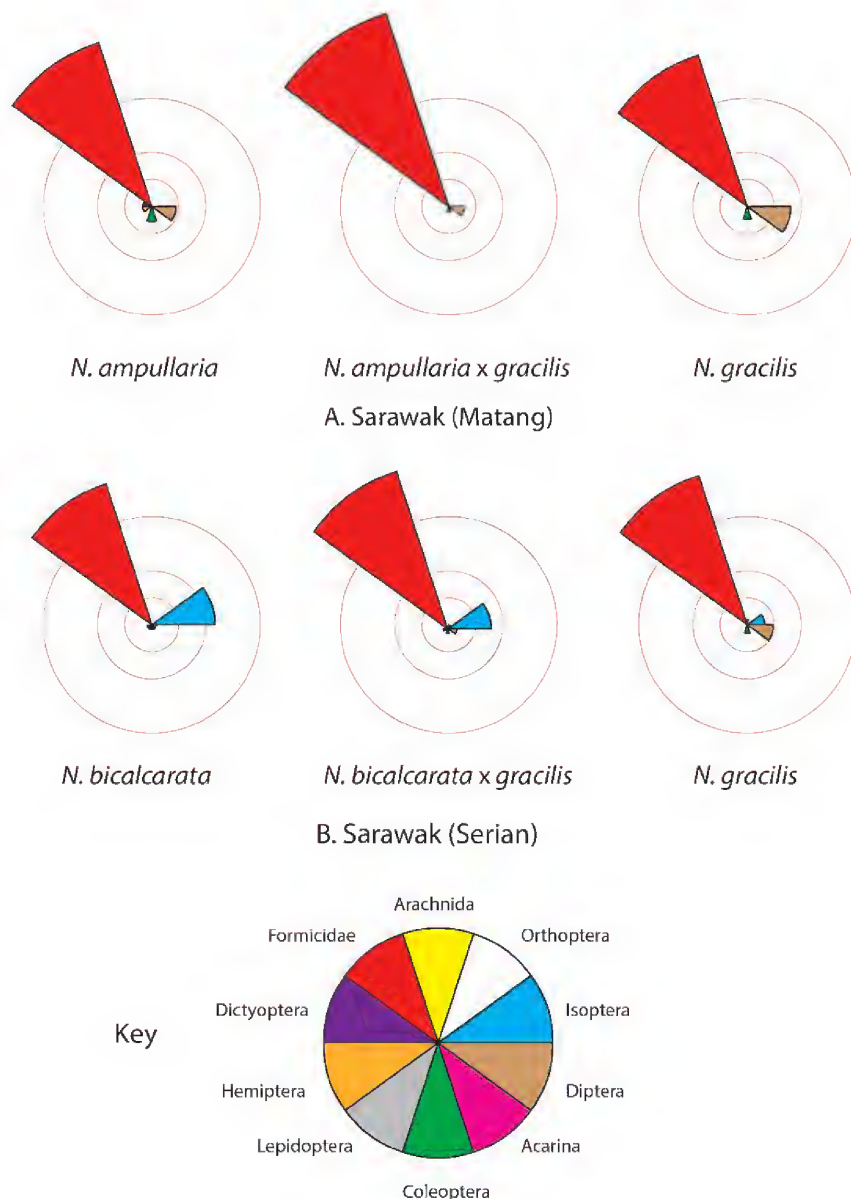


Figure 3: Prey capture patterns (with prey resolved to Order) in *Nepenthes* pitchers at (A) Matang, and (B) Serian, in Sarawak.

pitchers, all of which play important roles in pitcher function, include the large thorns that project down from the base of the pitcher lid (these thorns are actually giant nectaries (Merbach *et al.* 1999)), the wide peristome, the absence of a waxy zone on the inner surfaces of the pitchers, and swollen, hollow pitcher tendrils (Fig. 1B). The thorns provide a source of nectar for *Camponotus schmitzi* ants, which have a mutualistic association with this species (Clarke & Kitching 1995; Bonhomme *et al.* 2010). These ants often wait for prey under the overhanging peristome, and nest in the hollow tendrils. Thus, the physical characteristics of *N. bicalcarata* pitchers demonstrate a high level of specialization towards this mutualism, in which the plant benefits from having its pitchers maintained and prey capture rates enhanced by *C. schmitzi*, in return for providing the ants with food and domicile (Clarke & Kitching 1995; Thornham *et al.* 2012). In *N. bicalcarata* \times *N. gracilis*, the thorns are reduced to small lumps (it is not yet known whether these contain functional nectaries), the peristome is narrow, there is a well-developed waxy zone (which could be difficult for *C.*

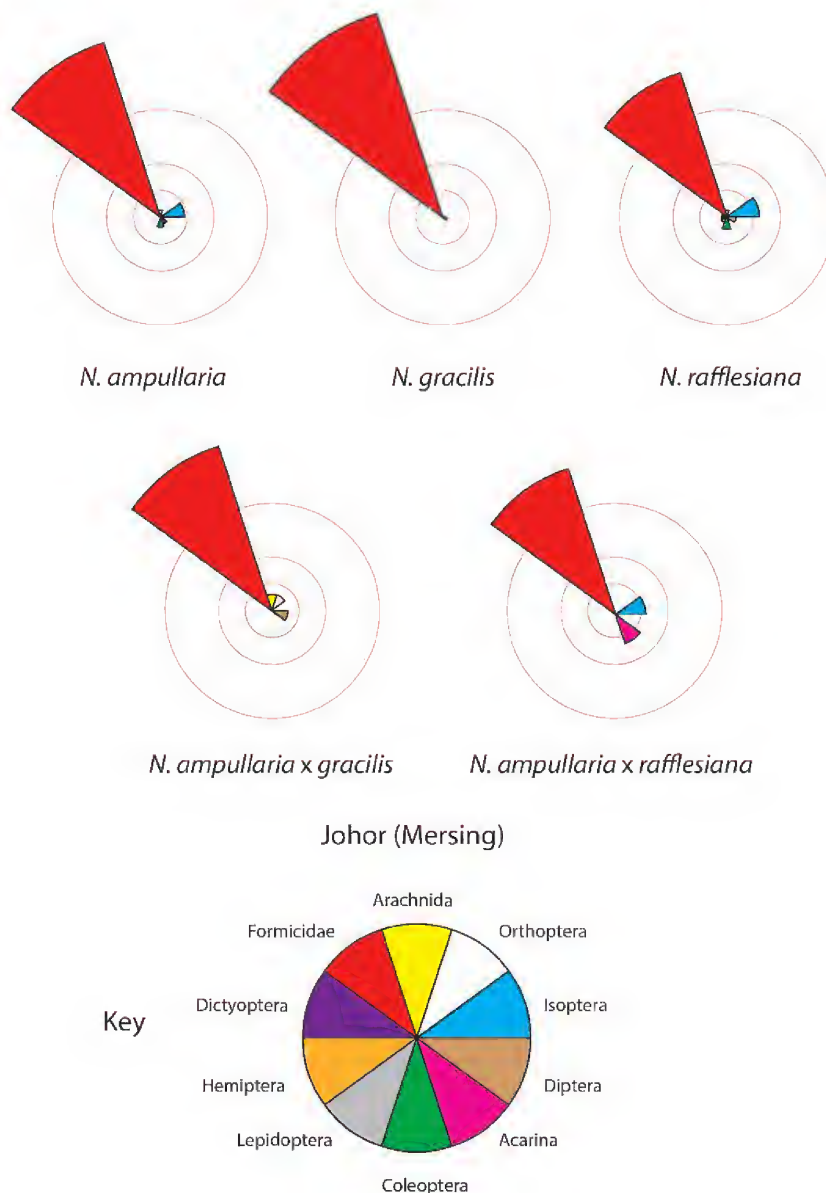


Figure 4: Prey capture patterns (with prey resolved to Order) in *Nepenthes* pitchers at Mersing, in Johor.

schmitzi workers to traverse), and the tendrils lack the cavities which *C. schmitzi* nest in. Therefore, the mutualism between *N. bicalcarata* and *C. schmitzi* cannot exist in this hybrid, because all of the unique traits of *N. bicalcarata* pitchers that facilitate it are either lost or unable to function as they do in *N. bicalcarata*.

On the face of it, *N. bicalcarata* × *N. gracilis* pitchers appear to be more similar to those of its other parent species, *N. gracilis*, in that they have a fairly narrow, cylindrical shape, a clearly defined waxy zone, a narrow peristome and solid tendrils (Fig. 1C). These characteristics are also reasonably representative of the pitchers of many *Nepenthes* species that are assumed to be unspecialized with regards to prey capture, such as *N. mirabilis* and, until recently, *N. gracilis*. However, *N. gracilis* has also been shown to possess a specialized trapping mechanism (Bauer *et al.* 2012b). In this species, the broad pitcher lid has a complex array of wax crystals on its lower surface (similar to those of the waxy zone inside the pitchers) and the thin, flexible nature of the lid enables it to “flick” back and forth when it is gently struck by surrounding objects, or rain

drops. This causes insects that are feeding at nectaries on the lower surface of the lid to be flicked into the pitcher cavity, where they drown and are digested by highly acidic pitcher fluids (Chou *et al.* 2014). By contrast, the lids of *N. bicalcarata* × *N. gracilis* pitchers are thicker and more rigid than those of *N. gracilis* (presumably, this makes them less flexible). It is yet not known whether they possess the complex array of wax crystals on their lower surfaces, or whether they are capable of functioning in the same way as those of *N. gracilis*. If all of the specialized characteristics of *N. bicalcarata* and *N. gracilis* pitchers are also lacking (or operate less effectively) in the hybrid, then by implication, the hybrid pitchers will be unspecialized and are unlikely to be able to obtain supplementary nutrients as effectively as the parent species. This does not mean that hybrid pitchers will fail to trap arthropod prey – virtually any pitfall that contains fluid will trap and retain some arthropods – it simply indicates that the hybrid appears to be less effective at doing so than its parents.

Whether or not hybrids trap enough prey to be able to reproduce effectively remains to be seen, but the evidence from *ad hoc* field observations suggests that for whatever reason, hybrid *Nepenthes* rarely persist in the wild for long periods. There are no known examples of any putative hybrid swarms that show evidence of becoming reproductively stable. There has been some conjecture among biologists that *N. × kinabaluensis* (= *N. rajah* × *N. villosa*) exists as a stable, independent population on Mt. Kinabalu, but there is no evidence of recruitment of new individuals into this swarm via sexual reproduction. Indeed, the only immature plants in the area belong to one of the parent species, *N. villosa*, substantially undermining any arguments for reproductive independence of *N. × kinabaluensis* from at least one of its parent species. Although hybrid *Nepenthes* are fertile, and we have regularly seen pollen collected from hybrid male flowers by a variety of pollinators, examples of fertilization of female hybrid flowers are very rare. At Matang in mid-2010, we observed many female inflorescences of *N. ampullaria* and *N. gracilis* plants, all bearing large numbers of fruits, but only one female inflorescence of *N. ampullaria* × *N. gracilis*, which appeared to bear just one fruit (Fig. 1H). If this pattern is representative, then a lack of recruitment of new individuals (in the absence of introgression with their parents), coupled with apparently low rates of fertilization of female flowers, indicate that while hybrids may be capable of trapping enough prey to produce inflorescences, there may be significant barriers to gene exchange among hybrid plants in the wild, meaning that it is more likely that their pollen will end up on the stigmas of a parent species than on those of another hybrid, or that any pollen that reaches the stigmas of a hybrid inflorescence will probably have come from a parent species as well. Thus, it appears more likely that the primary contribution by hybridization to diversification in *Nepenthes* is to facilitate gene flow between species via introgression.

The potential contribution of introgression to diversification in *Nepenthes* becomes apparent when the role of climate in the distributions of specialized and unspecialized *Nepenthes* are considered. Bauer *et al.* (2012a) and Moran *et al.* (2013) demonstrated that the so-called “dry type” pitcher format (i.e., cylindrical pitcher with a well-developed waxy zone and a narrow peristome) is found throughout the range of the genus, whereas the more specialized “wet type” (i.e., broader pitcher with reduced/no waxy zone and a broad peristome) is largely confined to perhumid habitats in the Sunda region. All of the highly specialized species from Borneo and Sumatra that have been detected to date grow in mixed populations with at least one other species of *Nepenthes*, and usually that species has the dry type pitcher format (C. Clarke, pers. observ.). In general, species whose pitchers conform to the dry type format appear to be less specialized (although there are some notable exceptions, such as *N. albomarginata* and *N. campanulata*), but tolerant of greater variation in environmental parameters than species that utilize the wet type trap format.

In undisturbed habitats, reproductive isolation prevents gene flow between co-occurring species with different trap formats. However, when disturbances occur that are significant enough to disrupt flowering seasons, interspecific gene flow can be facilitated via hybridization followed by introgression. This increases the gene pool of specialized species, which presumably has some negative effect on the effectiveness of its specialization, but highly specialized nutrient sequestration strategies appear to be very sensitive to habitat disturbances (Chin *et al.* 2010), and may break down anyway. Prolonged introgression could effectively “shift” a specialized species away from specialization, and towards a more generalized prey capture strategy. In highly disturbed and unpredictable habitats, this is likely to be beneficial, as undisturbed habitats are the stronghold of specialists, but disturbed and unpredictable habitats are the stronghold of generalists. This may be why we see so many hybrids in recently-disturbed sites in Borneo. If the vegetation at these sites is allowed to recover, the hybrids die out and introgression effectively ceases, but if the disturbances continue, then so does hybridization and introgression. Thus introgression appears to be a mechanism to promote gene flow among *Nepenthes* species during disturbances or periods of environmental unpredictability, making them more resilient, but less specialized.

Conclusion

The role of hybridization in the evolution and diversification of *Nepenthes* remains hypothetical, and this study serves only to show that the amount and variety of prey caught by three common lowland hybrids is less than, or equal to, that of their parent taxa. This could be a consequence of loss (or reduction) of specialized pitcher structures in hybrids that have one or more parent species that target specific types of prey using highly modified pitchers. Detailed manipulative experiments are required to investigate the ecological fitness of hybrid *Nepenthes* in the wild, and we hope that this study will somehow contribute to these.

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GERMINATING *NEPENTHES* SEED: PUTTING MYTHS TO REST

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Keywords: cultivation, *Nepenthes*, seed, gibberellic acid, heat mat, dark period.

Introduction

The Internet is full of personal accounts and guides on how to grow *Nepenthes* from seed. However, there is a plethora of misinformation available. Many of these myths involve the treatment of *Nepenthes* seeds before they are sown. In particular, whether they require a dark period in order to induce germination. A period of darkness after being sown has been shown to increase germination in other genera of plants such as *Cyclamen* (Ross & Ellis 2014), but there has been no conclusive study whether *Nepenthes* require a dark period to germinate. Others swear that treating seeds with gibberellic acid (GA3) can aid in the germination of *Nepenthes* seed. Again, no conclusive study has been published on this claim. The final claim is that sowing seeds and putting the pot on top of a heat mat will increase germination. Again, no study has been published legitimizing this claim. This study is an attempt to put many of these myths to rest. We hypothesize that none of the treatments will yield better results than the control treatment.

Materials & Methods

The experiment was replicated three times using seed from a horticultural hybrid created using a female *Nepenthes* 'Rokko' being grown at North Carolina State University and pollen from a male *Nepenthes veitchii* (Pink) from Malesiana Tropicals being grown by Bob Harrel. Mason McNair harvested the seed in early December 2013 by allowing the pods on the mother plant to open naturally into a paper envelope. The seeds were sorted by Mason McNair into lots of 50 seed and then mailed to Jeremiah Harris to be treated and sown. The six treatments in this experiment consisted of an untreated control, a heat mat treatment, a gibberellic acid treatment, and three dark period treatments. The gibberellic acid treatment consisted of soaking the seeds for 12 hours in 250 ppm of gibberellic acid (GA3). The heat treatment consisted of placing each pot of seeds onto a heat mat set for 21°C for the duration of the experiment. The three dark treatments consisted of leaving the pots in a closet for a designated period of time after the seeds were sown. For the first dark treatment, the seeds were in the dark for 4 days before being taken to the greenhouse with the rest of the treatments. For the second dark treatment, the seeds were in the dark for 7 days before being taken to the greenhouse. For the final dark treatment, the seeds were in the dark for 10 days before being taken to the greenhouse. All seeds, including the untreated control, were sown onto sterilized long-fibered sphagnum. Each treatment consisted of 50 seeds. The seeds were checked for germination weekly from the date of being sown until the conclusion of the experiment. All seeds were treated and sown on January 16, 2014. The experiment took place in a naturally lit greenhouse that is kept at 24-27°C during the day, 13-16°C at night, and 70-95% humidity. No artificial lighting was used for this experiment. Data was collected over 22 weeks.

Germination of *N. 'Rokko'* x *N. veitchii* (Pink)

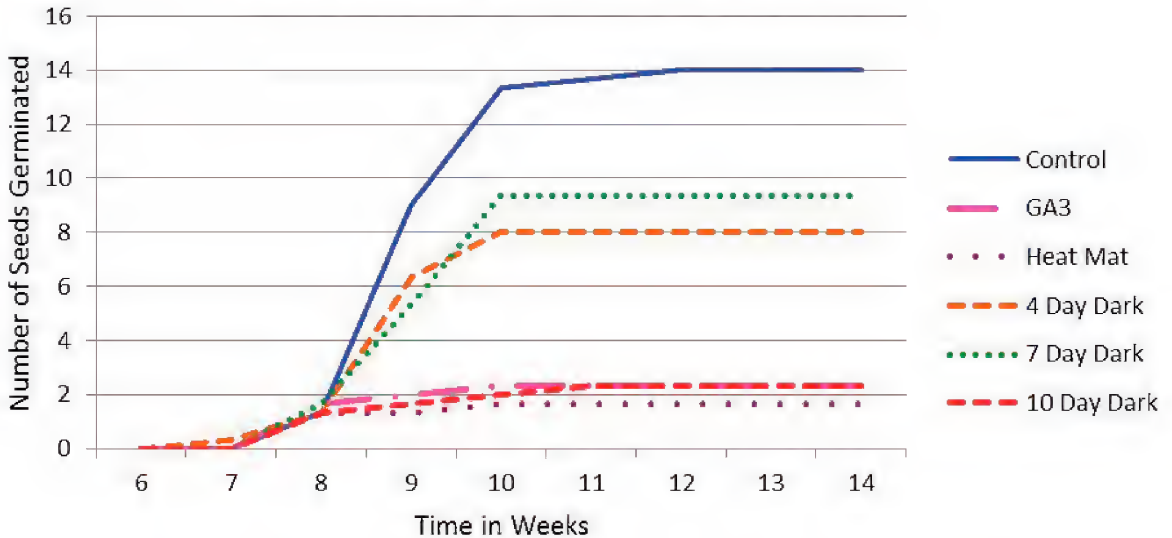


Figure 1: Average total number of seeds that germinated over a period of 14 weeks for each treatment. First germination did not occur until week 7. Data represent the mean of 3 replications.

Data & Results

The average time between sowing seeds and first germination was seven and a half weeks. Maximum germination was achieved by the twelfth week after sowing the seeds. After the twelfth week some treatments saw a slight decrease in plant survival; however, these plant deaths were insignificant for the purpose of this study. The control treatment had the best germination of all of the treatments, followed by the seven-day and four-day dark period treatments (Fig. 1). The GA3 treatment, heat mat treatment, and 10-day dark period treatment appeared to be inhibitive to seed germination.

In order to achieve the best germination for *Nepenthes*, seed should be sown as soon as possible after harvest directly onto fresh media.

Conclusion

The results of this experiment show that using a heat mat, treating seeds with gibberellic acid, or providing a dark period can be detrimental to *Nepenthes* seed germination. It is possible that others may reach different conclusions from personal, at home experiences. The lack of germination in the initial 7 weeks after sowing implies there is some sort of warm stratification needed for successful *Nepenthes* seed germination. Future research is needed to confirm this however. In the future, we suggest people ignore the many myths found on the Internet regarding growing *Nepenthes* from seed and instead sow their seed onto fresh media. We suggest long-fibered sphagnum (LFS), peat moss, a mix of peat moss and perlite, milled LFS, or a mix of milled LFS and peat moss for successful seed germination. In future experiments, we hope to repeat our treatments using true lowland and true highland species seed, artificial lighting, and a range of different media.

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RESOLUTION OF THE RELATIONSHIPS WITHIN THE NORTH AMERICAN PITCHER PLANT GENUS, *SARRACENIA*

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The North American pitcher plants (*Sarracenia*) are arguably one of the most charismatic groups of carnivorous plants and have garnered a great deal of attention for their diversity in trapping structures. The first *Sarracenia* specimen was described as early as 1570, and since then, the relationships among the species have remained an enigma for researchers. Originally, there were several attempts at describing relationships among *Sarracenia* through morphological measures, but with little consensus. The first genetic studies did little to improve our understanding of the relationships within *Sarracenia*, but did identify *Darlingtonia californica* (Cobra Lily) as the most ancient of the Sarraceniaceae family, with *Heliamphora* sharing a recent common ancestor with *Sarracenia* (Albert *et al.* 1992; Bayer *et al.* 1996; Neyland & Merchant 2006). The most recent genetic approach attempted to uncover species relationships using eight genes (Ellison *et al.* 2012). While Ellison *et al.* (2012) provided some resolution within the genus, most species relationships remained a mystery; however, they were able to date the genus to roughly 0.5-3 million years old, which is extremely recent in evolutionary time. In addition, members of the *Sarracenia* genus are prone to rampant hybridization with one another, making it even more difficult to determine the relationships among species.

Building off of these previous studies, we captured 199 genes across the 11 species recognized by Mellichamp and Case (2009) (*alabamensis*, *alata*, *flava*, *jonesii*, *leucophylla*, *minor*, *oreophila*, *psittacina*, *purpurea*, *rosea*, and *rubra*), along with three subspecies/varieties from the *purpurea* complex, two subspecies from the *rubra* complex, one *minor* variety, and two *flava* varieties (Stephens *et al.* 2015). These data were analyzed in a way that takes different genes' history into account, allowing them to elucidate many relationships with the genus (Fig. 1). In accordance with previous genetic attempts, *oreophila*, *alata*, *leucophylla*, and the *rubra* complex are closely related (Ellison *et al.* 2012; Neyland & Merchant 2006). However, the *rubra* complex remains ambiguous, as the relationships of the subspecies are not well supported. Additionally, *alata* does not appear to be a genetically distinct species and may be considered a part of the *rubra* complex. Interestingly, results seem to support the species level designation of *alabamensis* (*rubra* subsp. *alabamensis*) and *jonesii* (*rubra* subsp. *jonesii*), which have important implications regarding their status as endangered species. Both are found in isolated populations (Fig. 2) and are very morphologically different from other members of the *rubra* complex.

Contrary to previous genetic attempts at resolving the relationships within the genus, the *purpurea* complex is not basal to all other *Sarracenia*. Rather, this complex shares a recent common ancestor with *psittacina/flava/minor*. *Sarracenia purpurea* is the most widespread *Sarracenia* species, ranging from the Coastal Plain across Canada (Fig. 2). The complex has generally been divided into two subspecies, with one subspecies having three varieties. These designations are based on geographic distribution (Fig. 2). The results suggest that *purpurea* subsp. *venosa* var. *montana* is the ancestral form of *purpurea*, which has implications for its conservation status. Specifically, this species is under consideration for listing as an endangered taxon by the U.S. Fish and Wildlife Service and the Convention on International Trade in Endangered Species (CITES). The remaining *purpurea* subspecies relationships are less supported. Finally, the relationships found between *minor*, *psittacina*, and *flava* were supported by previous studies (Bayer *et al.* 1996; Neyland & Merchant 2006) with Ellison *et al.* (2012) supporting the sister relationship of *flava* and *psittacina*.

**MP-EST Species tree,
199 nuclear loci**

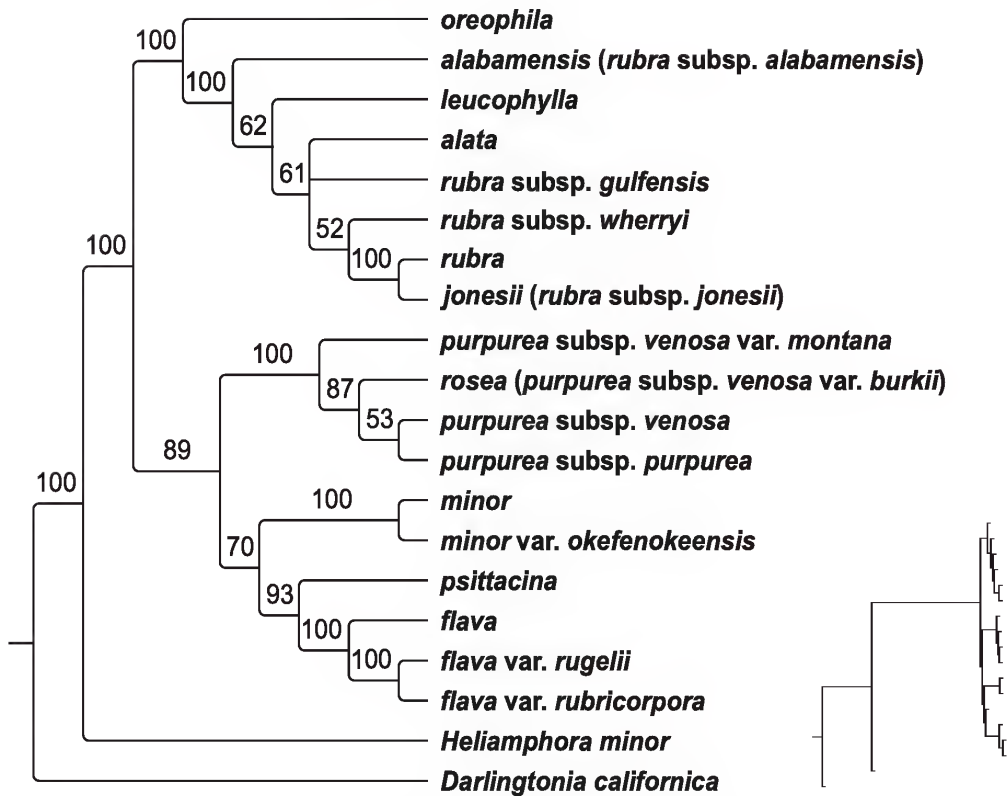


Figure 1: *Sarracenia* species tree based on 199 nuclear genes. Bootstrap support is listed above respective nodes. Bootstraps of >85 are well supported relationships, 70-85 are mildly supported, and <70 are considered weakly supported. Nodes with <50 support are collapsed (i.e. *alata* and *rubra* subsp. *gulfensis*).

This latter relationship is noteworthy, considering that these two species display opposite extremes of pitcher morphology.

In the context of biogeography, some interesting trends emerge. First, *oreophila* and *purpurea* subsp. *venosa* var. *montana* are both found on ancient soils in the Appalachians (Fig. 2). This combined with their placement as the most ancient species in each of their respective groups might suggest that *Sarracenia* ancestors may have originated in that region. This hypothesis may also explain the current geography of descendants of the *oreophila* ancestor (*alabamensis*, *leucophylla*, *alata*, and some *rubra* subspecies) along the Gulf Coastal Plain (Fig. 2), with the idea that descendants gradually migrated down the Apalachicola-Chattahoochee River drainage. Likewise, descendants from the group that are more closely related to *purpurea* subsp. *venosa* var. *montana* (*purpurea* subsp. *venosa*, *purpurea* subsp. *purpurea*, *minor*, *psittacina*, and *flava*) may have migrated down drainages that lead to the Atlantic Coastal Plain (Fig. 2). The other possible scenario is that the descendants from the Appalachians migrated down the Apalachicola-Chattahoochee River drainage and diverged east and west of the drainage. Future work focusing on the population genetics of *Sarracenia* can better elucidate the geographic origin and spread of this group. This is of interest, especially in regard to the *rubra* and *purpurea* complex.

The use of 199 genes has greatly improved our understanding of *Sarracenia* evolution. Resolving these relationships has important implications for conservation especially given the confusion in nomenclature designations (Ellison *et al.* 2014). For example, the listing of *S. rubra* subsp. *ala-*

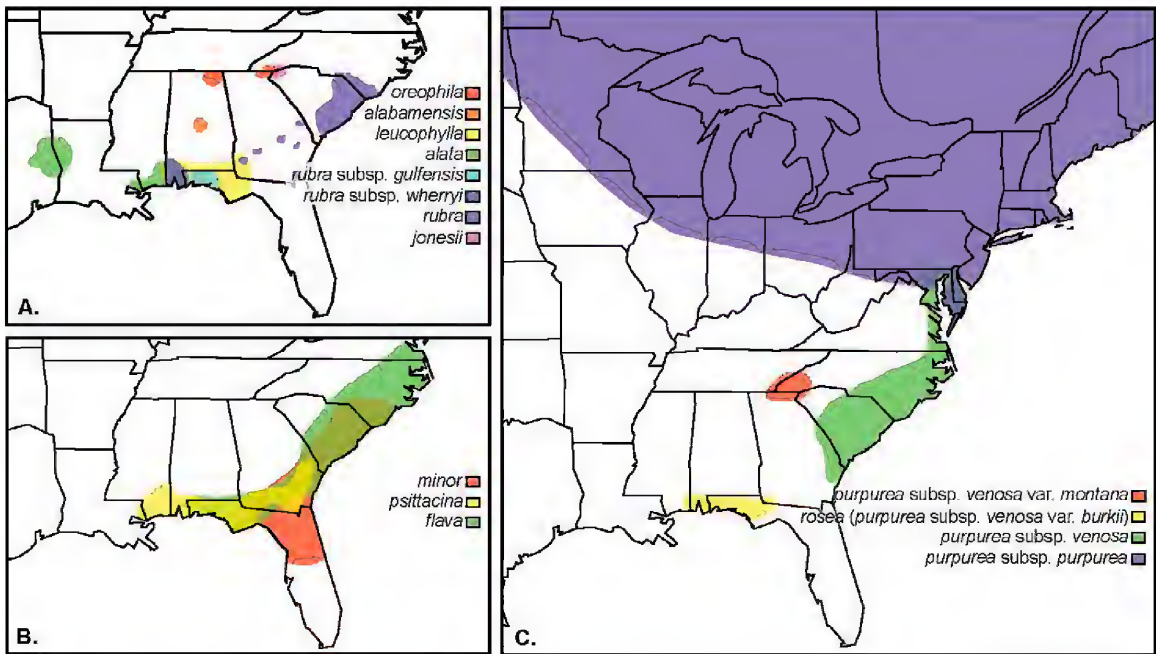


Figure 2: Range maps for *Sarracenia* species. (A) Species and subspecies ranges from the *oreophila* clade. (B) Ranges for the *flava*–*minor*–*psittacina* clade; varieties are not shown. (C) Ranges of the subspecies within the *purpurea* complex.

bamensis to *S. alabamensis* can have profound influences on resources allocated to its conservation. Therefore, an important next step is to reevaluate the nomenclature across the genus in lieu of the resolved evolutionary relationships, with specific emphasis on the *rubra* and *purpurea* complex. This will hopefully lead to less confusion for management and conservation officials that are in charge of protecting these rare and endangered species.

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CULTIVATION OF AUSTRALIAN AQUATIC INSECTIVOROUS PLANTS

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I have been growing aquatic *Utricularia* since 2009, and have always been lucky in how well they grow; it seems they are more adaptable than one would initially think. Last year just prior to the 2014 ICPS conference in Cairns, I offered to show some local sites to a few of the attendees, one site was my collection, the rest wild. During the drive from my collection to the conference, my passenger, Darren Cullen, suggested I do a quick article on one of the ponds I use, thinking this would be far too short and dull I decided to expand to a basic cultivation guide.

I have always been inquisitive, resulting in the death of many a plant, but all in the name of curiosity and improvement, so in my opinion justified. This is a very concise and basic summary of the main methods I have utilised and learnt over the years to better grow my collection.

I will start with what aquatic carnivores I will be covering, *Utricularia aurea*, *australis*, *gibba*, *stellaris*, and *Aldrovanda*. I also use *Utricularia uliginosa*, *bifida*, *limosa*, *dichotoma*, and *monanthas* as affixed aquatics at depths of 1-120 cm, although I have recorded wild *uliginosa* as deep as 2.3 m in the wild and have no doubt they would grow deeper, providing the irradiance was supplied.

All will grow together in the one pond, tank, or dam, so the size is not a major issue. I have grown *U. gibba*, *uliginosa*, and *bifida* in containers as small as 100 ml, and setup new dams with local variants of all but *Aldrovanda*. All will grow in a depth from 1 cm to 2 m happily, providing other conditions are right, *bifida* must be attached to timber or the substrate, but the rest are happy as suspended aquatics, including *U. uliginosa* which is at home amongst *U. gibba* clumps.

For a standard 2- or 3-foot tank, a 3-5 cm layer of your chosen substrate is added, I prefer what is labelled as premium potting mix and *Typha* leaves, as it is cheap, effective, and long lasting. Then add a 2-4 cm layer of clean sand as an anchoring base for companion plants as well as to hold down the initial substrate. I have tried various grain sizes and found it makes no real difference.

The water is then added carefully; then let the tank settle for a minimum of 3 days, or ideally 2-3 weeks to help create a stable environment, allow any particles to settle, and any chemicals to mix in.

Table 1.	
Summary of a standard 1 m tank	
Water depth	1-200 cm
Water pH	5.6-8.7 (optimum is 6.2-6.6)
Water temperature	10-42 °C
Substrate in order of success	<div>1. sphagnum moss (2-3 cm)</div> <div>2. Premium potting mix and <i>Typha</i> leaves (3-5 cm)</div> <div>3. premium potting mix (2-4 cm)</div> <div>4. pine bark chips (3-4 cm)</div> <div>5. <i>Typha</i>/grass/reed leaves (2-3 cm lightly compressed)</div> <div>6. peatmoss (2-3 cm)</div> <div>7. clay (5-6 cm as this relies on companion plants)</div> <div>All covered by a thin 2-4 cm layer of sand or gravel as anchorage for companion plants/<i>Utricularia</i> and to hold down substrate.</div>
Irradiance	<div>Full sun all day, full morning sun and bright light.</div> <div>50% shade cloth receiving full sun all day seems to be optimal.</div>

Table 2.

Accompanying plants		
<i>Baloskion tetraphyllum</i> , <i>Baumea</i> , <i>Chorizandra</i> <i>cymbaria</i>	Pipe grass	These three species work well in dams, ponds in excess of 100 L, or potted in small tanks/ponds. They allow anchorage and decaying matter adds to acidity and nutrients of the water.
<i>Eleocharis</i>	Pincushion grass	Only suitable for shallow setups less than 10 cm deep. Alternatively attractive in a pot that is raised, certain species are fast spreading, others slow. Brilliant bog garden plants, easy germinating, not too competitive and attractive.
<i>Marsilea</i>	Nardoo	Thrives in depths of 1-40 cm, can get out of control and choke out <i>Utricularia</i> if not controlled in smaller setups, an array of attractive specimens exist in the <i>Marsilea</i> group.
<i>Nelumbo</i>	Lotus lily	Limited to larger setups such as dams or large ponds, although they form clumps are rather spaced and well suited for aquatic carnivores to grow at the bases of. Also as a stolonous species release higher levels of CO ₂ .
<i>Nymphaea</i>	waterlily	Best in ponds deeper than 40 cm, but small species work well in depths as low as 5 cm, attractive and aid in protection from birds and strong sun in open setups, may get out of control and need to be removed bi- to tri-annually.
<i>Nymphoides</i>	False waterlily	Although typically much faster growing than <i>Nymphaea</i> , <i>Nymphoides</i> die off faster, resulting in less competition, and are usually smaller, they are the water lily of choice in small setups.
<i>Ottelia ovalifolia</i>	Swamp lily	Again smaller than most <i>Nymphaea</i> , attractive, offers avian protection and anchorage. These plants are happy in 5 cm to 2 m deep water.
<i>Phragmites australis</i> , <i>Juncus</i>	reed	Best suited to larger setups, can be a nuisance if not potted and confined. I also like to cut off inflorescences as the seeds are easily spread and readily germinate.
<i>Potamogeton</i>	Pond weed	Can get out of control, certain species are preferable due to varied growth rates. These plants are used for aesthetics and anchorage. They are happy in a 5 L bowl to a 100,000 L lake.
<i>Typha domingensis</i>	Bull rush	Depths of 0-150 cm, easily contained in solid pots, long pots work best, their stolonous nature also adds to the CO ₂ and plants provide shade and wind protection
<i>Vallisneria australis</i>	Water grass	Aesthetics is the only reason I use this plant, predominantly in glass aquarium setups, it is the proffered nursery for small shrimp and fish.
<i>Pistia stratiotes</i>	Water lettuce	In confined aquarium only, not in open ponds due to risk of spreading; periodically removed to prevent overcrowding; (removed plants must burnt), extreme care must be taken with this species and it should only be grown in a container with a lid or indoors. The hanging roots provide breeding habitat for prey such as daphnia and allow the plants to absorb massive amounts of nutrients. The divisional rate of these plants can be used to predict the nutrient level of the water.

Table 2. Continued.		
Top three hazardous plants		
<i>Hydrilla</i>		Can easily choke out <i>Utricularia</i> and <i>Aldrovanda</i> , hard to remove from a collection, in my opinion unattractive.
<i>Lemna</i>	Duck weed	Although this plant acts in a similar manor to <i>Pistia</i> , in that it is excellent at absorbing nutrients, they mat the surface and block all light. <i>Lemna</i> is also extremely hard to eradicate and quickly spreads to any water vessel, in the case of greenhouse invasions to water trays, into bromeliads and even across flooded bog gardens.
<i>Hymenachne amplexicaulis</i>	Hymanachne	Fast growing, easy spreading, as a result chokes out other flora.
Fauna		
Unknown	Horn snail	Small snails full grown at 4-5 mm, these small species seem unable to consume plants and seem limited to algae
<i>Atyid shrimp</i>	Creek shrimp	Fast breeding, smaller specimen preyed on by <i>Utricularia</i> and <i>Aldrovanda</i> . Some are attractive and entertaining to watch
<i>Holthuisana</i>	Fresh water crab	Small freshwater crabs that thrive in tanks and ponds, they are preyed on by insectivorous plants when young, older crabs prey on tadpoles and toad-poles, two deadly threats when in large numbers
Dragon fly or damselfly		I allow the larvae to build up as the parents predate potential threats such as aquatic moth and mosquito and the young do no damage
<i>Ranata, Lethocerus</i>	Water scorpion	Smaller individuals provide prey, whilst larger specimen are entertaining to watch and predate potential threats
<i>Hydaticus and Macrogyrus</i>	Water beetles and skaters	Again provide food and predate potential threats
<i>Daphnia</i>		Provide food and help control algae
<i>Neosilurus, Bunocephalus, Pterygoplichthys.</i>	catfish	Like all fish they must only be used in small quantities, cat fish have an immense effect on algae and are a must have in ponds in excess of 100 L.
<i>Melanotaenia, Mogurnda, Oxyeleotris, Poecilia</i>	Rainbow fish, gudgeon, cod and guppy	These fish are predacious and attractive; their fry offer food for insectivorous plants. Rainbow fish and guppy must be controlled as they will rapidly form large colonies if not managed. Gudgeon and cod are slow moving and slow breeding predacious fish, for this reason they do little physical and chemical damage to ponds and tanks.
<i>Betta</i>	Fighter fish	These work well in setups between 5 and 20 L, any smaller and they typically pollute the water to fast, any larger and they seem uncomfortable, but they are happy as a solitary fish and predate anything they can fit in their mouth.
Faunal species to avoid in setups less than 200 L		
Turtles		Faecal matter destroys the water chemistry and their size and movements destroys breaks plants.
Goldfish, koi, and other carp		
Ells and larger fish species		



Utricularia uliginosa is happy to grow as a permanent attached aquatic.



Small children tubs such as these are perfectly suited to being a shallow ponds and deep water trays, also note the cut back *Typha*, this does not harm the plants and the leaves may be dried and utilised as substrate.

After this, companion plants may be added. They are not a necessity, but are highly recommended. Now after another week, the pH should be recorded, target pH is 6.2-6.6. If the pH is too high, add dried *Sphagnum* — it will float for a few days then sink and increase the acidity. Alternatively, peat tea may be used or dried sedges or grass leaves stuffed in a stocking. If the pH is too acidic, simply bucket out some water and replenish with new water. Once the pH is stable, the *Utricularia* are added. This same method may be adopted for large ponds. I use 1 cup of peat/sphagnum to 3 L; so a 250 L pond takes about 84 cups (or 8-9 compressed peat blocks), however for small tanks ½ to 1 cup per liter is



A large share tank at the back of my greenhouse for *Utricularia uliginosa*, *dichotoma*, *bifida*, *aurea*, *australis*, *gibba*, *stellaris*, and *Aldrovanda*, to the left is a poison pack converted to a CO₂ reactor.



Small bowls (8 L or less) are best used in greenhouses or collections where localities need to be separate; here *U. gibba*, *australis*, *uliginosa*, and *bifida* can all thrive.



Old bath tubs are common in sheds of rural regions and make excellent bog gardens and ponds.



A standard pot, the substrate is 3 cups of premium potting mix, pH of 8.0-8.2, yet *Utricularia aurea* thrives 8 months; not surprising as wild plants are common in heavily fertilised ditches in cropland.



A decorative pond containing *Aldrovanda*, *U. aurea*, and *U. gibba*.

enough, otherwise two 25 L bags of standard potting mix per 250 L. In the ball park estimates are fine.

For small setups (less than 100 L), I find the addition of a simple CO₂ reactor to be a great benefit to the growth rate. They are also simple to make and benefit all aquatic plants (however not recommended if there are fauna such as fish or crustaceans). Another benefit of a CO₂ reactor in an aquarium or pond is to increase acidity. I like to use a CO₂ reactor that is less than ¼ the size of the pond or tank as any larger and the water chemistry will decrease. My mix is one 7 g yeast sachet to 2 cups of white sugar in 5 L of water.

To counter algae, I use horn snail, this is a minute species that does not feed on plants and can be brought from some pet stores, alternatively or with them small catfish work well, especially in larger bodies of water (in excess of 100 L), as do freshwater mussels. In smaller ponds and aquariums, nitrogen levels can easily climb too high with fish.

Companion plants can be used to reduce algae growth by absorbing excess nutrients, lessening intense light, regulating the temperature, and also help *Utricularia* growth by adding CO₂ when older leaves decay or through stolon growth.

Now for a novelty, but rather effective method, we started to make some ponds, or in a couple cases cattle troughs using the old rear tires from tractors. We cement the base to make a watertight area and treat as any other pond. We put rocks around to hide them in the gardens. These and old bath tubs are easily obtained in rural regions and work brilliantly as large, low maintenance ponds.

GIANT *CEPHALOTUS* OF UNKNOWN ORIGINS

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Introduction

I have been growing *Cephalotus follicularis* for over 20 years. Initially, I was obsessed with growing specimen-type *Cephalotus* of different clones and to prove once-and-for-all that this was not a difficult plant to grow. Countless plants have met their demise as I experimented with various methods of cultivation. For those that have survived and flourished, I noticed one plant in particular that grew larger, more vigorous, and had a different pitcher/leaf morphology than *Cephalotus* 'Hummer's Giant' and the typical *Cephalotus*. However, I do not believe this plant to be just a better-grown specimen of 'Hummer's Giant'. Through the years, I have given and sold this plant to individuals calling it the "Bubble Giant", however, I have not received nor heard any feedback as to the well-being of those plants. So, for those reading this article and have received this plant from me, I would appreciate seeing some photos. For the remainder of this article, this plant will be referred to as the "unknown".

Origins

During my initial spark-of-entry into the hobby, I started collecting *Cephalotus* cuttings, plants, stems, and leaves from anyone who had the plant and was willing to give or sell a piece to me. Because of that activity, this plant is of an unknown origin because of the feverish pace by which I went about amassing what I had hoped would become a genetically diverse collection of plants. Where each of those contributors got their *Cephalotus* was not important to me at the time, so meticulous record keeping went to the wayside. However, the only one source I can claim to have received some of my materials from was Phil Mann. Other than that, one can only guess, because at that time (20+ years ago), the carnivorous plant hobby, in my opinion, was at its peak and almost every carnivorous plant enthusiast would have had a *Cephalotus* nestled in and amongst their collection.

Here lies my dilemma and the purpose of this article: to identify if this clone is currently in cultivation, and whether or not it has a given and accepted name, but has not yet been registered as a cultivar.

Characteristics

The most prominent features of this plant is its size, with foliar leaves measuring 6-11 cm and pitchers averaging 6 cm, but on rare occasions, can reach 10 cm long (Figs. 1-4). The rim of the largest pitchers produced on mature plants is approximately 2.5-3 cm wide. Another unique feature of this plant is the lid: being slightly wider than the rim and half as tall, it produces a semi-circular, half-ladle, parabolic shape. The vase of the pitcher (rim to base) is stouter, not as elongated as in the typical form, and the elegant "waist" just below the rim is not as pronounced, giving the pitchers an overall stunted appearance. This plant takes at least 5 years to reach maturity from mature leaf cuttings.

Cultivation

All of my plants are grown indoors under 4-foot, 40-watt fluorescent shop lights, 6 inches from the source. They are cultivated in an unheated, non-air-conditioned room with open windows where



Figure 1: A side-by-side comparison of 20+ year old plants. The “unknown” (left) and the typical form (right). Both growing in African violet mix in 6-inch pots.



Figure 2: A side-by-side comparison between the “unknown” (left) and ‘Hummer’s Giant’ (right). The foliar leaves of the “unknown” are much larger than the leaves on the ‘Hummer’s Giant’. Both plants are over 20 years old, cultivated under identical conditions, in 4-inch pots, and in African violet mix.



Figure 3: Pitcher comparison between 'Hummer's Giant' (left) and the "unknown" (center). While the "unknown" plant is green when cultivated under regular fluorescent 40-watt shop lights, the entire plant attains a rich pinkish-red coloration when grown under blue/red LED grow lights (right).

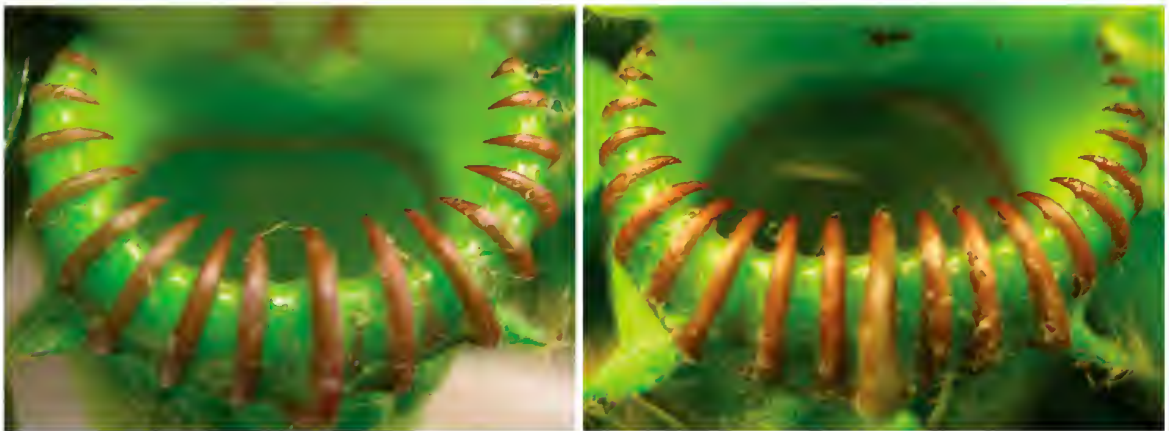


Figure 4: Pitcher rim comparison between 'Hummer's Giant' (left) and the "unknown" (right). While the comparison between the pitchers and rims may appear insignificant and such variations can be produced on the same plant, over 90% of the pitchers produced on either the "unknown" and 'Hummer's Giant' do conform to the photos.

they experience seasonal temperature changes of summer and winter. The lights are set on timers with a 16-hour photoperiod year round. Watering is by the tray method or with polyester wicks tucked into the pots' drainage holes, drawing water through capillary action from a water reservoir.

The potting media I use for my plants is an organic African violet mix from E.B. Stone Organics which contains fir bark, redwood compost, and sand; purchased from my local plant nursery. However, when propagating cuttings, I use a 50/50 peat and sand mix or pure perlite until roots and plantlets are evident. Then, they are transplanted into the African violet mix. Reproduction is by vegetative propagation (leaves and pitchers), division, and pups developing from side shoots.

NEW CULTIVARS

Keywords: cultivar, *Dionaea muscipula* ‘Spotted Hyena’.

Dionaea muscipula ‘Spotted Hyena’

Submitted: 21 March 2015

Dionaea ‘Spotted Hyena’ was found in a garden center in Germany in June 2013. The traps of are red and have darker and lighter spots on the inside and outside of the traps (Figs. 1 & 2). The main features of *Dionaea* ‘Spotted Hyena’ are, beside the eye-catching spots, the irregular teeth and uneven edges of the traps.

There are differences that separate *Dionaea* ‘Spotted Hyena’ from *Dionaea* ‘Spotty’.

- ‘Spotted Hyena’ is a flat-growing plant, whereas ‘Spotty’ is an upright-growing plant.
- The petiole of ‘Spotted Hyena’ is wider than the slim petiole of ‘Spotty’. Furthermore, the edges of ‘Spotted Hyena’ petiole are often brighter than its middle part.
- The edges of the traps of ‘Spotted Hyena’ are uneven with indentations and hillocks. The traps of ‘Spotty’ are proportional and smooth.
- The teeth of ‘Spotted Hyena’ are irregular in shape, length, and size and there are sub-teeth on some traps. The teeth of ‘Spotty’ are usual and average.

Vegetative propagation is required to maintain the characteristics of this cultivar.

The name, Spotted Hyena, is inspired by the African carnivore which is a strange and unusual beauty like this Venus flytrap.

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Figure 1: *Dionaea* ‘Spotted Hyena’ has darker and lighter red spots on the inside and outside of the traps, irregular teeth, and uneven edges.



Figure 2: The inside traps of *Dionaea* 'Spotted Hyena' have dark red spots (left) and the trap edges are uneven with irregular teeth (right).



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CARNIVOROUS PLANT CULTIVAR NAMES REGISTERED IN 2014

<i>Cephalotus</i> 'Dudley Watts' S.Morley, Pl.Carniv.35(2):42 (2014)	24.Jan.
<i>Dionaea</i> 'Kayan' G.Bily, Carniv.Pl.Newslett.43:26 (2014)	15.May
<i>Dionaea</i> 'Phoolan Devi' G.Bily, Carniv.Pl.Newslett.43:25 (2014)	15.May
<i>Dionaea</i> 'Sirius' C.Boiteux, Carniv.Pl.Newslett.43:135 (2014)	25.Dec.
<i>Dionaea</i> 'Sonic' Y.Sarzi, Carniv.Pl.Newslett.43:75 (2014)	01.Aug.
<i>Drosera</i> 'Portland Sunrise' J.Brittnacher & D.Crawford, Carniv.Pl.Newslett.43:27 (2014)	15.May
<i>Pinguicula</i> 'Rose' M.Ban, J.Insectiv.Pl.Soc.51:112 (2000)	15.Sep.
<i>Sarracenia</i> 'Black Jaw' S.Lascialfari, Carniv.Pl.Newslett.43:24 (2014)	15.May
<i>Sarracenia</i> 'Black Mamba' S.Ibelli, Carniv.Pl.Newslett.43:24 (2014)	15.May
<i>Sarracenia</i> 'Eva' H.Luhrs, Carniv.Pl.Newslett.43:137 (2014)	25.Dec.
<i>Sarracenia</i> 'Laughing Wizard' M.Hutley, Carniv.Pl.Newslett.43:107 (2014)	15.Sep.
<i>Sarracenia</i> 'Randy Rable' D'Amato, Carniv.Pl.Newslett.43:106 (2014)	15.Sep.
<i>Sarracenia</i> 'Silvia Luise' D.Richetti, Carniv.Pl.Newslett.43:23 (2014)	15.May
<i>Sarracenia</i> 'Vogel' H.Luhrs, Carniv.Pl.Newslett.43:136 (2014)	25.Dec.
<i>Sarracenia</i> 'Yellow Eel' S.Amoroso, Carniv.Pl.Newslett.43:74 (2014)	01.Aug.



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GREX NAMES PUBLISHED BY THE INTERNATIONAL *NEPENTHES* GREX REGISTRY

Gre x	Female parent	Male parent
Alien	<i>N. lowii</i>	<i>N. truncata</i>
Aristocrat	<i>N. lowii</i>	<i>N. aristolochioides</i>
Bargara	<i>N. talangensis</i>	<i>N. hamata</i>
Bella	<i>N. bellii</i>	<i>N. spectabilis</i>
Bello	<i>N. bellii</i>	<i>N. ampullaria</i>
Black Dragon	<i>N. izumiae</i>	<i>N. truncata</i>
Caesar	<i>N. merrilliana</i>	<i>N. truncata</i>
Courage	<i>N. “thorelii”</i> × <i>N. maxima</i>	<i>N. albomarginata</i>
Dangnoi	<i>N. mirabilis</i> var. <i>globosa</i>	<i>N. ampullaria</i> ‘Cantley’s Red’
Dragon Fire	<i>N. spectabilis</i>	<i>N. truncata</i>
Dragon Slayer	<i>N. sibuyanensis</i>	<i>N. truncata</i>
Exotic Dragon	<i>N. truncata</i>	<i>N. veitchii</i>
Exotic Lady	<i>N. maxima</i>	<i>N. talangensis</i>
Filipine Bells	<i>N. bellii</i>	<i>N. ventricosa</i>
Filipine Dragon	<i>N. bellii</i>	<i>N. truncata</i>
Firecracker	<i>N. albomarginata</i>	<i>N. veitchii</i>
Gothica	<i>N. sibuyanensis</i>	<i>N. maxima</i>
Inevitable	<i>N. rafflesiana</i>	<i>N. bicalcarata</i>
Jmaxeypiata	<i>N. eymae</i>	<i>N. ehippiata</i>
Laithai	<i>N. mirabilis</i> var. <i>globosa</i>	(<i>N. rafflesiana</i> × <i>N. ampullaria</i>) × <i>N. mirabilis</i>
Leviathan	<i>N. rajah</i>	<i>N. peltata</i>
Little Apple	<i>N. ampullaria</i> × <i>N. rafflesiana</i>	<i>N. mirabilis</i> var. <i>globosa</i>
Maka’ula	<i>N. alata</i>	<i>N. reinwardtiana</i>
Maximum	<i>N. maxima</i>	<i>N. mira</i>
Menehune	<i>N. ventricosa</i>	<i>N. campanulata</i>
Midtown	<i>N. eymae</i>	<i>N. </i> × <i>trusmadiensis</i>
Miss Stephanie	<i>N. eymae</i>	<i>N. jacquelineae</i> × <i>N. izumiae</i> ?
Odyssey	<i>N. lowii</i>	<i>N. veitchii</i>
Purple Deagon	<i>N. truncata</i>	<i>N. densiflora</i>
Red Leopard	<i>N. ventricosa</i>	<i>N. maxima</i>
Redneck	<i>N. truncata</i> × <i>N. “thorelii”</i>	<i>N. maxima</i>
Regal Dragon	<i>N. truncata</i>	<i>N. mira</i>
Spellbound	<i>N. bellii</i>	<i>N. spathulata</i>
Squat Dragon	<i>N. truncata</i>	<i>N. campanulata</i>
St. Brice	<i>N. alata</i>	<i>N. </i> × <i>mastersiana</i>
St. Phocas	<i>N. tobaica</i>	<i>N. truncata</i>
Striking Dragon	<i>N. truncata</i>	<i>N. stenophylla</i>
Sulawesi Dragon	<i>N. eymae</i>	<i>N. truncata</i>
Venturiana	<i>N. eymae</i>	<i>N. jacquelineae</i>
Xiriana	<i>N. mirabilis</i> var. <i>globosa</i>	<i>N. rafflesiana</i>

Notes:

In contrast to cultivars and Groups that are defined by phenotypic similarity, a grex (pl. greges or grexes) is a group of cultivated artificial hybrids defined by parentage.

For the time being the naming and registration of greges is only well established and sanctioned for orchids by the International Code of Nomenclature for Cultivated Plants (ICNCP). Nevertheless, a group of *Nepenthes* breeders (International *Nepenthes* Grex Registry, <http://nepenthes-grex-registry.org>) decided to name greges in this genus, following their own (non-ICNCP) rules.

The present listing (that will be updated as deemed fit) is not to be confused with our annual list of registered names of cultivars and Groups (that follow ICNCP standards).

In order to prevent confusion, the ICPS (being the International Registration Authority for the names of carnivorous plant cultivars and Groups) will from now on not allow grex epithets (as listed in the first column of the table above) as epithets or parts of epithets of new cultivars or Groups in the same denomination class (*Nepenthes*). This should allow coexistence of both naming systems and a possible future registration by the ICPS if grex naming will be sanctioned for non-orchid groups by the ICNCP.



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